Decreased Expression of CD3$^\zeta$ and Nuclear Transcription Factor $\kappa$B in Patients with Pulmonary Tuberculosis: Potential Mechanisms and Reversibility with Treatment

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Background. The protective immune response against Mycobacterium tuberculosis relies both on antigen-presenting cells and on T lymphocytes. In patients with different forms of tuberculosis, varying degrees of T cell function—ranging from positive delayed-type hypersensitivity, in asymptomatic infected healthy individuals, to the absence of the response, in patients with miliary or pulmonary tuberculosis (PTB)—have been reported. The decreased expression of CD3$^\zeta$ reported in T cells from patients with either cancer or leprosy has provided possible explanations for the altered immune response observed in these diseases.

Methods. The present study aimed to compare the expression of CD3$^\zeta$, nuclear transcription factor-$\kappa$B (NF-$\kappa$B), arginase activity, and cytokine production in 20 patients with PTB, in 20 tuberculin-positive asymptomatic subjects, and in 14 tuberculin-negative control subjects.

Results. Compared with those in tuberculin (purified protein derivative)–negative control subjects, peripheral-blood T lymphocytes from patients with active PTB had significantly decreased expression of CD3$^\zeta$ and absence of the p65/p50 heterodimer of NF-$\kappa$B. These alterations were reversed only in patients who responded to treatment. Also reported here for the first time is that the presence of arginase activity in peripheral-blood mononuclear-cell lysates of patients with PTB parallels high production of interleukin-10.

Conclusions. The presence of arginase could, in part, explain the decreased expression of CD3$^\zeta$. These findings provide a novel mechanism that may explain the T cell dysfunction observed in patients with PTB.

Tuberculosis (TB) is a leading cause of death due to infectious diseases worldwide [1]. In some areas where coinfection with HIV and Mycobacterium tuberculosis is common, TB rates have doubled during recent years, despite increased availability of antimycobacterial therapy. Protective immunity against mycobacterial infection is considered to be a cell-mediated process in which macrophages, T cells, and, possibly, natural killer cells mediate the inhibition of mycobacteria [2–4]. Studies of immune dysfunction in TB have reported changes in lymphocyte subset ratios [5, 6], diminished responses to mycobacterial antigens [7, 8], altered cytokine-production patterns [9, 10], and altered macrophage activation and antigen presentation [11, 12]. Furthermore, the suppression of T cell responses by mycobacterial infections might be mediated by the increased production of soluble factors, including interleukin (IL)–10 or transforming growth factor–$\beta$ [13, 14]. Alterations in the expression of CD3$^\zeta$ and other T cell signal-transduction proteins have been reported in cases of cancer [15–17], leprosy [18], autoimmune...
diseases [19, 20], and HIV [21] and could partially explain the T cell dysfunction seen in patients with these diseases. The mechanisms leading to these alterations are poorly understood. However, previous reports have described that, in tumors, the amino acid l-arginine plays a central role in the regulation of T cell function [22, 23]. Elsewhere we have reported that the depletion of l-arginine modulates CD3ζ expression and T cell function in activated human T lymphocytes [24]. Therefore, to determine whether M. tuberculosis infection could alter T cell function, the present study investigated the expression of CD3ζ and nuclear transcription factor xB (NF-xB) in patients with active pulmonary TB (PTB), in tuberculin (in the form of purified protein derivative [PPD])–positive asymptomatic subjects, and in PPD-negative healthy control subjects. In addition, the present study also investigated in these 3 groups the correlation between T cell dysfunction and depletion, by arginase, of l-arginine in plasma. The results of the present study may indicate a novel mechanism by which M. tuberculosis evades the immune response.

PATIENTS, MATERIAL, AND METHODS

Study patients and subjects. After signed informed consent (previously approved by the Institutional Review Board of the Louisiana State University [LSU] Health Science Center) had been obtained, 30 mL of peripheral blood was drawn from each of (1) 20 patients with active PTB as defined by clinical symptoms, radiological findings consistent with TB, and bacteriological confirmation; (2) 20 PPD-positive asymptomatic subjects; and (3) 14 healthy PPD-negative control subjects attending the LSU-Wetmore TB clinic at the LSU Health Sciences Center in New Orleans. Table 1 shows demographic data for each of the 3 groups. Of the 20 PPD-positive asymptomatic subjects, 6 (Hispanic or Asian) presented with bacille Calmette-Guérin scars. Patients with PTB were newly diagnosed, had not received previous anti-TB treatment, and tested negative for HIV. Patients with hepatitis, malnutrition, immunodeficiency, other active infectious diseases, cardiac complications, immunosuppressive therapies, or cancer were excluded from the study. Successful treatment was defined as the disappearance of the clinical signs and symptoms of infection with M. tuberculosis, the resolution of the radiological changes associated with TB, and sputum culture that was negative for PTB. Of the 20 patients with PTB, 10 were reevaluated and tested after 4 months of treatment; an additional 5 months of therapy was required for 3 of these 10 patients.

For the intradermal Mantoux test, 0.1 mL of 5 tuberculin units of PPD tuberculin (Connaught) was injected intradermally on the anterior part of the left forearm. The test was read 48 h after the injection. Induration of ≥5 mm was considered to be a positive reaction.

Cell preparation. Samples of venous blood (30 mL) were diluted 1:3 in Hanks’ balanced salt solution and were separated over Ficoll-Paque (Pharmacia Biotech). Peripheral-blood mononuclear cells (PBMCs) enriched in T cells were obtained by use of T cell–enrichment columns (R&D Systems). The enriched populations were ≥90% CD3+ T cells that were used for electrophoretic mobility–shift assay (EMSA) and reverse-transcriptase polymerase chain reaction (RT-PCR).

Flow cytometry. For the detection of CD3ζ, 1 × 10⁶ PBMCs were stained, for 15 min at 4°C, with 1 µg of either anti–CD3–fluorescein isothiocyanate (clone UCHT1) or isotype control (Beckman-Coulter). The cells were washed and incubated, for 8 min at 4°C, in PBS containing digitonin at 500 µg/mL (Wako BioProducts) plus 2.5 µg of anti–CD3ζ–phycoerythrin (clone 2H2D9) antibody (Beckman-Coulter). Then the cells were washed, resuspended, and analyzed immediately in a Coulter-EPICS flow cytometer (Beckman-Coulter). A similar protocol was used for the detection of CD4ζ (clone RPA-T4) and CD8ζ (clone HIT8a) (Beckman Dickinson).

Cytoplasmic- and nuclear-extract preparation. Cytoplasmic extracts were obtained by lysing 3 × 10⁶ PBMCs in 0.5% Triton X-100 (pH 7.5) and protease inhibitors, as described elsewhere [18]. The lysates obtained were either analyzed immediately, to test for arginase activity, or frozen at −70°C. T cell nuclear extracts were prepared as described elsewhere [25]. In brief, the nuclei were lysed in 25 µL of nuclear buffer and

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Age, mean (range), years</th>
<th>Sex</th>
<th>Race</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>PPD negative (14)</td>
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<td>6</td>
<td>8</td>
</tr>
<tr>
<td>PPD positive (20)</td>
<td>40 (23–69)</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>PTB (20)</td>
<td>39 (26–59)</td>
<td>14</td>
<td>6</td>
</tr>
<tr>
<td>Total (54)</td>
<td>28</td>
<td>26</td>
<td>26</td>
</tr>
</tbody>
</table>

* Hispanic or Asian; all 11 had bacille Calmette-Guérin scars (none of the white or African Americans subjects had such scars).
then were kept frozen, at −70°C, for the purpose of further analysis of NF-κB by EMSA. Protein concentration in both types of extract was determined by use of a BCA protein assay kit (Pierce Chemical).

**EMSA.** Samples (2 μg) of nuclear extracts from 6 of the PPD-negative control subjects, 14 of the PPD-positive asymptomatic subjects, and 14 of the patients with PTB were preincubated, for 10 min at room temperature, in gel shift–binding buffer and 0.5 μg/mL poly-dIdC. Then, 1 μL of 32P-labeled (50,000 cpm/μL/sample) (Perkin Elmer Life Sciences) NF-κB consensus oligonucleotide (Santa Cruz Biotech) was added to the reaction mixture, and the solution was incubated for 20 min. DNA-protein complexes were resolved by electrophoresis on 6% polyacrylamide gels, were dried, and then were autoradiographed by use of BIOMAX-MR (Kodak) film at −70°C.

**RT-PCR.** Total RNA from 2 × 10^6 T cells from 6 of the PPD-negative control subjects, 14 of the PPD-positive asymptomatic subjects, and 14 of the patients with PTB that was extracted by use of TRIzol (Invitrogen), was treated with DNase I (Invitrogen), and was reverse-transcribed by use of Superscript II (Invitrogen). PCR amplification was performed by use of CD3ζ and β-actin primers published elsewhere [19]. Each primer (0.25 μmol/L) and 1 U of Taq DNA polymerase in PCR buffer containing MgCl₂ (2.5 mmol/L), dNTP (0.4 mmol/L), and 2 μCi of 32P α-dCTP (Perkin Elmer) were added to 2-μL samples of cDNA. For CD3ζ, 25 PCR cycles were run, at 96°C for 30 s, then at 55°C for 40 s, and then at 72°C for 1 min; for β-actin, 27 PCR cycles were run, at 95°C for 1 min, then at 54°C for 1.5 min, and then at 72°C for 1.5 min.

**Arginase-activity assay.** Arginase activity was determined as described elsewhere [26]. In brief, cell lysates were added to 25 μL of Tris-HCl (50 mmol/L; pH 7.5) containing MnCl₂. This mixture was heated at 55°C–60°C for 10 min; then 150 μL of carbonate buffer (100 mmol/L; Sigma) and 50 μL of L-arginine (100 mmol/L; Sigma) was added, and the solution was incubated at 37°C for 20 min. The reaction from L-arginine to L-ornithine was detected, at 515 nm, after ninhydrin solution had been added and the solution had been incubated at 95°C for 1 h.

**Plasma levels of L-arginine.** Levels of L-arginine in plasma samples from the 14 PPD-negative control subjects and the 20 patients with PTB were determined by use of high-performance liquid chromatography, as described elsewhere [27]. In brief, the plasma samples were deproteinized and derivatized, and then 50 μL of each sample was tested. A standard curve with known concentrations of L-arginine was run for each experiment.

**Proliferation assay.** PBMCs from 6 of the PPD-negative control subjects, 10 of the PPD-positive asymptomatic subjects, and 10 of the patients with PTB that were stimulated with PPD (10 μg/mL) were plated at 1 × 10^5/well and were cultured at 37°C for 48 h. A 0.5-μCi portion of 3H-thymidine (Perkin Elmer) was added to each well, and the wells were incubated for an additional 18 h, at 37°C. Each condition was run in triplicate. Cells were lysed by freezing and thawing, were har-
vested onto a Unifilter-96 GF/B (Packard), and were counted by use of a TOPcount Microplate Scintillation Counter (Packard).

Cytokine production. To determine the possible role that Th2 cytokines play in the induction of arginase, samples of PBMCs from 6 of the PPD-negative control subjects, 14 of the PPD-positive asymptomatic subjects, and 14 of the patients with PTB were cultured, for 48 h, in RPMI-1640 (Cambrex) in the presence of either 10 μg/mL PPD (Statens Seruminstitut, Copenhagen, Denmark) or anti-CD3 (30 ng/mL) plus anti-CD28 (100 ng/mL). The supernatants were tested for human IL-2, interferon (IFN)–γ, IL-4, and IL-10, by use of ELISA (Biosource). Samples of unstimulated PBMCs were used as controls.

Statistical analysis. Intergroup comparisons were performed by use of either Student’s t test or 2-way analysis-of-variance using the Graph-Pad statistical program (Prism 3.0; Graph-Pad). The association between CD3ζ and arginase was estimated by use of a regression model and Pearson’s correlation coefficient. P ≤ .05 was considered to be significant.

RESULTS

Diminished expression of CD3ζ-chain in T cells from patients with PTB. Expression of CD3ζ was tested in samples of PBMCs from 14 PPD-negative control subjects, 20 PPD-positive asymptomatic subjects, and 20 patients with PTB. The upper panel of figure 1 shows representative scatter plots comparing the expression of CD3ζ and CD3ε in samples of PBMCs from a PPD-negative control subject, a PPD-positive asymptomatic subject, and a patient with PTB; expression of CD3ζ in the patient with PTB was lower than that in either the PPD-negative control subject or the PPD-positive asymptomatic subject—a decrease that was not due to a decrease in the number of T cells, because the expression of CD3ε was similar (range, 76%–85%) in all 3 of the groups. The lower panel of figure 1 shows that the mean fluorescence intensity (MFI) of CD3ζ was significantly (P < .001) lower in the patients with PTB (mean ± SD, 14.35 ± 2.5) than in the PPD-negative control subjects (mean ± SD, 28.55 ± 1.2), and similar and significant decreased expression of ζ-chain was also observed in CD4 and CD8 T cell subsets. Of the 20 PPD-positive asymptomatic subjects, 4 (20%) had markedly decreased expression of CD3ζ, although none of these 4 had signs or symptoms of TB.

Reexpression of CD3ζ to normal levels after successful treatment of patients with PTB. Of the 20 patients with PTB who were receiving anti-TB therapy, 10 were retested for expression of CD3ζ. As summarized in table 2, 7 of these 10 patients responded to treatment after 4 months, with expression of CD3ζ (MFI, 28.5) having recovered to levels similar to those in the PPD-negative control subjects; in contrast, the other 3 of these 10 patients did not respond to treatment and had decreased expression of CD3ζ, which recovered to normal levels after additional treatment.

Diminished expression of CD3ζ in patients with PTB—not due to decreased expression of CD3ζ mRNA. RT-PCR was used to investigate whether the decreased expression of CD3ζ in patients with PTB was the result of a decrease in expression of CD3ζ mRNA. The levels of CD3ζ mRNA were normalized by use of β-actin as internal control. Representative results of the integrated densitometry values of the ratio between expression of CD3ζ and expression of β-actin, in PPD-negative control subjects (3/6), PPD-positive asymptomatic subjects (4/14), and patients with PTB (5/14), are shown in the bar graph in figure 2, where this ratio is expressed as a percentage. Analysis comparing the expression of CD3ζ mRNA and of β-actin mRNA failed to show significant intergroup differences in the expression of CD3ζ mRNA (P = .81).

Decrease in nuclear expression of p65 of NF-κB in T cells from patients with PTB. NF-κB plays an important role in T cell activation after antigenic stimulation. After freshly isolated T cells had been activated with anti-CD3 plus anti-CD28 antibodies, nuclear extracts were obtained and were tested, by EMSA, for NF-κB. Figure 3 shows representative EMSA data for 5 PPD-negative control subjects, 5 PPD-positive asymptomatic subjects, and 5 patients with PTB. The expression of both the p65/p50 heterodimer and the p50/p50 homodimer of NF-κB was observed in T cells from the 5 PPD-negative control subjects (lanes 1–5) and the 5 PPD-positive asymptomatic subjects (lanes 6–10); in contrast, T cells from 4 of the 5 patients with PTB (lanes 11, 12, 14, and 15) lacked heterodimer p65/p50, expressing only homodimer p50/p50. Data from all patients and controls are summarized, in tabular format, under

Table 2. Reexpression of CD3ζ in patients with pulmonary tuberculosis who responded to treatment

<table>
<thead>
<tr>
<th>Patient</th>
<th>Pretreatment</th>
<th>4 months</th>
<th>9 months</th>
<th>Response</th>
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<tr>
<td>1</td>
<td>8.3</td>
<td>28.1</td>
<td></td>
<td>R</td>
</tr>
<tr>
<td>2</td>
<td>2.4</td>
<td>1.9</td>
<td>26.7</td>
<td>NR-R</td>
</tr>
<tr>
<td>3</td>
<td>2.9</td>
<td>30.1</td>
<td></td>
<td>R</td>
</tr>
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<tr>
<td>7</td>
<td>10.3</td>
<td>16.5</td>
<td>32.1</td>
<td>NR-R</td>
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<td>8</td>
<td>2.9</td>
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<td>NR</td>
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<tr>
<td>10</td>
<td>9.8</td>
<td>7.6</td>
<td>29.3</td>
<td>NR-R</td>
</tr>
</tbody>
</table>

a The mean intensity fluorescence for control subjects was 28.5.

b NR, nonresponder; R, responder; NR-R, patients who did not respond (i.e., were classified as NR) during the first 4 months of treatment but who did respond (i.e., were classified as R) after additional treatment.
Figure 2. Detection of CD3ζ mRNA in T cells by reverse-transcriptase polymerase chain reaction. Representative samples from 3 tuberculin (purified protein derivative [PPD])–negative subjects, 4 PPD-positive asymptomatic subjects, and 5 patients with pulmonary tuberculosis (PTB) are shown. In all cases, β-actin was used as an internal control. Densitometry analysis (by analysis of variance), using the ratio between expression of CD3ζ and expression of β-actin, did not show statistically significant intergroup differences in expression of CD3ζ mRNA.

the gels in figure 3. The T cells of 5 (36%) of the 14 patients with PTB who were tested and of 13 (93%) of the 14 PPD-positive asymptomatic subjects who were tested expressed the p65/p50 heterodimer of NF-κB. Interestingly, all of the patients with PTB who lacked NF-κB p65 (pretreatment) also had decreased expression of CD3ζ—and heterodimer p65/p50 was restored after successful treatment (data not shown).

Correlation between elevated levels of arginase and low expression of CD3ζ in PBMCs from patients with PTB. In vivo, levels of L-arginine are tightly regulated by arginase. Therefore, we tested the levels of arginase in PBMC lysates from patients with PTB. As shown in figure 4A, arginase activity was significantly elevated in patients with PTB (mean, 127.8 ± 88.1 nmol [P = .002]) and in PPD-positive asymptomatic subjects (mean, 22.67 ± 28.4 nmol [P = .024]), compared with that in PPD-negative control subjects (mean, 3.03 ± 3.3 nmol). As shown in figure 4B, statistical analysis using Pearson’s correlation coefficient revealed that, in the 20 patients with PTB, there was a highly significant (P < .001) inverse association (r = −0.8256) between decreased expression of CD3ζ and high arginase activity as measured by the conversion of L-arginine to L-ornithine. As shown in figure 4C, the levels of L-arginine in the plasma of patients with PTB (mean, 88 ± 7 μmol/L) were significantly less (P = .004) than those in control subjects (mean, 153 ± 16 μmol/L).

Alteration of cell proliferation and cytokine production in patients with PTB. In response to stimulation with PPD, PBMCs from patients with PTB had a significantly lower proliferation rate than did those from PPD-negative control subjects (P = .002) and PPD-positive asymptomatic subjects (P = .007), as shown in figure 5C; the rate was not different between the PPD-negative control subjects and the PPD-positive asymptomatic subjects. As shown in figures 5A and 5B, in the presence of PPD the PBMCs did not produce IFN-γ and shifted to a predominantly down-regulatory IL-10 response, especially in the PPD-positive control subjects and the patients with PTB. In addition, these cells did not produce either IL-2 or IL-4 (data not shown). In light of these findings, a test was performed to determine whether the inability, by PBMCs from PPD-positive control subjects and from patients with PTB, to produce Th1 cytokines was specific to stimulation with mycobacterial antigens; stimulation of PBMCs by anti-CD3 plus anti-CD28 abolished the effect of mycobacterial antigens, and, as seen in figure 5A, the cells were able respond normally, indicating that T cell unresponsiveness in patients with TB is antigen specific and related to mycobacterial antigens.
Figure 3. Electrophoretic mobility-shift assay using nuclear transcription factor (NF–κB) consensus oligonucleotides and nuclear extracts of T cells from 6 tuberculin (in the form of purified protein derivative [PPD])–negative control subjects, 14 PPD-positive asymptomatic subjects, and 14 patients with pulmonary tuberculosis (PTB). To determine translocation of NF–κB to the nucleus, T cells were stimulated for 1 h in the presence of 30 ng/mL anti-CD3 plus 100 ng/mL anti-CD28. Representative data for 5 PPD-negative control subjects, 5 PPD-positive asymptomatic subjects, and 5 patients with PTB show that heterodimer p65/p50 was absent from T cells from most of the patients with PTB but was present in most of the PPD-negative control subjects and PPD-positive asymptomatic subjects. Homodimer p50/p50 was expressed consistently in all groups. NF–κB dimers are indicated by arrows. The tabular data below the gels summarize the results for the 3 groups.

### DISCUSSION

A large body of clinical and experimental evidence has shown that the level of immune competence varies among patients with mycobacterial diseases. The different clinical presentations of TB appear to be associated with the degree of alteration of the immune response against the mycobacteria. The present study has shown that patients with active PTB (1) have a significant decrease in the expression of CD3ζ, which is an important signal-transduction protein in T cell activation, and (2) lack the p65/p50 NF–κB heterodimer, which is important in the activation of certain T cell genes. The data suggest that these alterations could in part explain the loss of a protective immune response against *M. tuberculosis*, and they may help us to understand the mechanisms that lead to T cell dysfunction in TB.

The results of the present study show that, in a high percentage of patients with PTB, the expression of CD3ζ is significantly lower than that in PPD-negative control subjects. Interestingly, although the PPD-positive asymptomatic subjects studied did not show any signs of detectable active disease, a small percentage (20% [4/20]) also showed decreased expression of CD3ζ, suggesting that some individuals might develop alterations in the expression of signal-transduction proteins during latent infection due to *M. tuberculosis*. Therefore, it will be important to continue to monitor these PPD-positive asymptomatic subjects, to determine whether these signal-transduction changes precede the development of the active disease; in addition, similar decreased expression of CD3ζ was observed in both CD4+ and CD8+ T cell subsets. The decreased expression of CD3ζ has been reported in infectious diseases such as leprosy and AIDS and at the site of mycobacterial infection in TB [18, 21, 28]. As has clearly been seen in patients with leprosy [18], changes in the expression of CD3ζ and p56lck and the absence of NF–κB p65 in the nucleus are more predominant in patients with lepromatous leprosy than in either patients with tuberculous leprosy or healthy control subjects, and therefore the alterations seen in those patients might be a reflection of immunological incompetence. Likewise, the signal-transduction alterations seen in patients with PTB may reflect both the different stages of immune competence and T cell–function impairments that may result in the induction of anergy [13], as is commonly seen in miliary TB.

Nuclear transcription factors such as NF–κB appear to play a central role in both T cell activation and the regulation of certain cytokine genes [29]. The present study found that the 64% of patients with PTB who lacked the p65/p50 heterodimer...
Expression of CD3\(z\) in Pulmonary TB

Figure 4. A, Cytoplasmic extracts of peripheral-blood mononuclear cells (PBMCs) from 14 tuberculin (in the form of purified protein derivative [PPD])–negative control subjects, 20 PPD-positive asymptomatic subjects, and 20 patients with pulmonary tuberculosis (PTB), which were tested for arginase activity measured in terms of conversion of L-arginine to L-ornithine. PBMCs from patients with PTB produced more arginase \((P = 0.02)\) than did those from healthy control subjects. B, Analysis by Pearson’s correlation coefficient, showing negative correlation \((r = -0.8256)\) between arginase activity and expression of CD3\(z\) in PBMCs from patients with PTB. C, Levels of L-arginine, which were significantly higher \((P < 0.004)\) in plasma from PPD-negative control subjects than in that from patients with PTB.

also had decreased expression of CD3\(z\), suggesting that it is possible, although not yet proven, that the T cell–receptor signal via CD3\(z\) could be a determining factor in the release of NF-\(\kappa B\) p65 from its inhibitor, I\(\kappa B\), and in its subsequent translocation to the nucleus to initiate cell activation and cytokine-
gene regulation. Therefore, a more definitive understanding of these interactions in TB will require both an extensive analysis of more patients and testing for other transcription factors also known to play important roles in cytokine-gene regulation.

The mechanisms leading to the decreased expression of CD3\(z\) in disease are poorly understood. In vivo, levels of L-arginine are regulated, in great part, by a balance between arginase and inducible nitric oxide (NO) synthase, 2 enzymes using L-arginine for the production of L-ornithine and NO, respectively \([22, 30]\). NO is an important cytotoxic mechanism in mac-

Figure 5. A and B, Cytokine production by peripheral-blood mononuclear cells (PBMCs) from tuberculin (in the form of purified protein derivative [PPD])–negative control subjects, PPD-positive asymptomatic subjects, and patients with pulmonary tuberculosis (PTB), stimulated with either PPD \((10 \mu g/mL)\) or anti-CD3 plus anti-CD28 and then measured by ELISA of supernatants after 48 h in culture. C, T cell proliferation, which, after stimulation with PPD for 48, was significantly decreased \((P = 0.002)\) in patients with PTB. Unstimulated (NS) PBMCs from all 3 groups were used as controls in the experiments.
rophanes, whereas l-ornithine is a precursor of polyamines essential for cell proliferation [31, 32]. Arginase can be induced in macrophages by a variety of cytokines, including IL-4, IL-13, and IL-10 [33] and transforming growth factor–β [34], as well as by lipopolysaccharides [35]. In murine peritoneal macrophages, stimulation with IL-4 plus IL-13 induces arginase production that rapidly reduces levels of l-arginine, resulting in the induction of T cell dysfunction [36]. We recently have reported that, in the absence of l-arginine, stimulated T cells have both decreased CD3ζ expression and low proliferation, observations similar to those is seen in patients with cancer and other infectious diseases [24].

The high levels of arginase seen in the PBMCS of patients with PTB correlates with decreased expression of CD3ζ and contrasts with the low arginase levels and normal expression of CD3ζ that were seen in the PPD-negative control subjects; therefore, arginase may play a role in the metabolic regulation of plasma levels of l-arginine in infected individuals. The results of the present study also show that levels of l-arginine in patients with PTB were significantly lower than those seen in the PPD-negative control subjects—allowing one to speculate that these low levels may contribute to decreased expression of CD3ζ, as has been seen in T cells cultured in the absence of l-arginine [24]. Although the number of samples tested in the present study is small and although definitive conclusions therefore cannot be drawn, the overall results suggest that patients with PTB produce high levels of arginase that deplete levels of l-arginine, which, in turn, induces T cell dysfunction characterized by decreased cell proliferation, increased production of IL-10, and decreased production of both IL-2 (data not shown) and IFN-γ. It is unclear which cells are producing arginase—or which signals trigger the increase in arginase production—in patients with PTB; however, it is possible that mycobacterial infection of macrophages and/or increased IL-10 production may be responsible for this event.

Preliminary data show that patients responding to anti-TB therapy reexpress all of the signal-transduction proteins, including CD3ζ and NF-κB p65. The recovery of these proteins could be due to different factors, such as decrease in either the antigenic burden or the inflammatory response (i.e., IL-10), increase in Th1-type cytokines, alternative macrophage activation, or decrease in the levels of arginase. The findings of the present study also suggest that the reexpression of signal-transduction molecules may benefit the clinical outcome in patients, because those patients who did not respond to treatment maintained diminished expression of CD3ζ and NF-κB and showed no signs of an improved immune response. In addition, preliminary observations show that these patients still have high levels of arginase activity and high production of IL-10. However, further testing of a larger number of patients, along with a more complete follow-up, will be necessary to confirm these findings.

In summary, the present study found that a significant number of the patients with PTB who were studied had alterations in the expression of several T cell signal-transduction proteins, alterations that were similar to those observed in patients with cancer or other diseases. The similarity of T cell signal-transduction alterations in diseases with different pathophysiological characteristics suggests the possibility that a common mechanism causes such changes. The presence of increased levels of arginase in macrophages of patients with PTB suggests that this enzyme plays a role in regulation of the immune response, in light of the fact that high levels of arginase play an important role in both the down-regulation of CD3ζ and the induction of T cell dysfunction. These observations of molecular and functional characteristics in TB may provide new tools to study and monitor patients, to determine how these characteristics effect the development of immune dysfunction, and to study new pathways to block suppressor mechanisms. This endeavor would reestablish that the function of the immune system combined with anti-TB therapy will benefit the clinical outcome in patients with TB.

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References


