

Low Number of Peripheral Blood B Lymphocytes in Patients with Pulmonary Tuberculosis

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The cellular immune response plays a critical role in the containment of persistent *Mycobacterium tuberculosis* infection; however, the immunological mechanisms that lead to its control are not completely identified. The goal of this study was to evaluate B (CD19+) and T (CD3+) peripheral blood lymphocyte profiles and T-cell subsets (CD4+ and CD8+) in patients with pulmonary tuberculosis (TB). Percentages ($p = 0.02$) and absolute numbers ($p = 0.005$) of B cells were significantly lower in patients with pulmonary TB than in healthy donors. In contrast, percentages ($p = 0.12$) and absolute numbers ($p = 0.14$) of T cells were similar in TB patients and healthy donors. No significant differences in percentages of CD4+ ($p = 0.19$) or CD8+ ($p = 0.85$) T cells between patients and healthy donors were observed. In summary, patients with pulmonary tuberculosis had a lower number of peripheral blood B lymphocytes than healthy controls.

Keywords B lymphocytes, T lymphocytes, Tuberculosis.

INTRODUCTION

The immune response plays a critical role in the containment of persistent *Mycobacterium tuberculosis* (*M. tuberculosis*) infection (Aaron et al., 2004). However, the immunological mechanisms that lead to its control are not

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completely identified (Kaufmann, 2006). It is known that innate immunity is very important in tuberculosis (TB), macrophages and natural killer cells are decisive (Raja, 2004). But that control requires multiple underlying immune responses resulting in the generation of protective lymphocyte and cytokine production to stop *M. tuberculosis* growth within the lungs (Doherty and Andersen, 2005).

There is substantial evidence supporting the major role of CD4+ T cells in containing tuberculosis at all stages of the disease (Jones et al., 1997), and animal models support the suggestion that CD4+ T cells are the most important aspect of the protective response in primary TB infections (Rahemtulla et al., 1991). The main function of CD4 T cells is cytokine production and immunity against mycobacterial infection by means of a Th1 response (Ferraz et al., 2006). In addition, CD8+ T cell also contribute to a successful immune response against *M. tuberculosis* by producing IFN- γ and causing lysis of infected cells (Stenger, 2001). CD8+ T cells are thought to become more important in the later phases of the disease (Serbina and Flynn, 2001). The role of B cells in combating *M. tuberculosis* infection is less clear, and there are only a few studies explaining their function in TB. There are reports that involve B cells in developing pulmonary granuloma formation during mycobacterial infections; however, the mechanisms involved remain unidentified (Bosio et al., 2000). The aim of this study was to analyze the distribution of peripheral lymphocyte subpopulations of B and T cells from patients with pulmonary tuberculosis.

MATERIAL AND METHODS

Patients

Patients suffering from pulmonary tuberculosis (n = 17; Table 1) were diagnosed based on clinical, radiological, and bacteriological data obtained by the medical staff of the Department of Health of the state of Sonora, following international criteria. Individuals that were HIV-positive, pregnant, patients suffering from cancer, alcoholics and drug abusers were excluded. Patients were 22 to 77 years old (mean 57.9, median 59); 9 patients were men and 8 were women. All patients were treated following the posologic scheme prescribed by the Mexican Ministry of Health. The healthy control group comprised 17 asymptomatic volunteers (Table 1), 23 to 75 years old (mean 50.6, median 51), 9 were men and 8 were women, without previous history of tuberculosis. All patients and volunteers were vaccinated with BCG at birth. Patients and healthy volunteers presented a positive skin reaction to intradermal PPD injection of 5.7 ± 3 mm, and 10 ± 4.0 mm, respectively. Weak PPD response from patients was considered anergy. All subjects signed a letter of

Table 1: Characteristics of enrolled subjects.

	TB Patients	Healthy donors
Total	17	17
Age, years		
Mean	57.9	50.6
Median	59	51
Range	22 to 77	23 to 75
Sex		
Female	8	8
Male	9	9
IDR, mm (mean)	5.7 ± 3	10 ± 4

consent to participate in the protocol that was approved by the ethics committee of the University of Sonora and the corresponding state health authorities, following international regulations.

Isolation of PBMC and Flow Cytometry Analysis

Peripheral blood mononuclear cells (PBMC) were isolated by Histopaque-1077 (Sigma Chemical Co., St Louis, MO), gradient centrifugation, and 5×10^5 cells were stained with a combination of monoclonal antibodies (mAbs) as follows: to analyze B and T cells, double labeling was performed using anti-CD19FITC (clone 4G7) and anti-CD3PerCP (clone SK7). To evaluate T cell subpopulations (CD4+ and CD8+), triple labeling, using anti-CD4FITC (clone SK3), anti-CD8PE (clone SK1) and anti-CD3PerCP, was performed. For isotype controls, cells were stained with Simultest γ_{2a} FITC/ γ_1 PE.

All mAbs and isotype controls were from BD Biosciences (San Jose, CA, USA). Incubations were done for 15 minutes at room temperature in the dark, followed by two washes with PBA [phosphate-buffered saline (PBS) containing 0.1% bovine serum albumin (BSA) (Research Organics, Cleveland, OH) and 0.01% sodium azide]. Cells were fixed with PBA containing 1% paraformaldehyde (PFA) and data was acquired with a fluorescence activated cell sorter FACScalibur® (BD Biosciences). Ten thousand events were computed and analyzed with the CellQuest® software (BD Biosciences). To analyze the percentages of B and T cells, lymphocytes were defined and gated on a dot-plot of forward scatter (FSC) versus side scatter (SSC) (Fig. 1a) and dot plot of CD19-FITC versus CD3-PerCP was made (Fig. 1b). Measurement of T cells subpopulations (CD4+ and CD8+) was done within a gate on a dot-plot of CD3-PerCP versus side scatter (Fig. 1c) and dot plot of CD4-FITC versus CD8-PE was made (Fig. 1d). To calculate the total number of cells, samples from subjects were analyzed using a Beckman Coulter hematology blood analyzer (ActT diff), and cell percentage data were used.

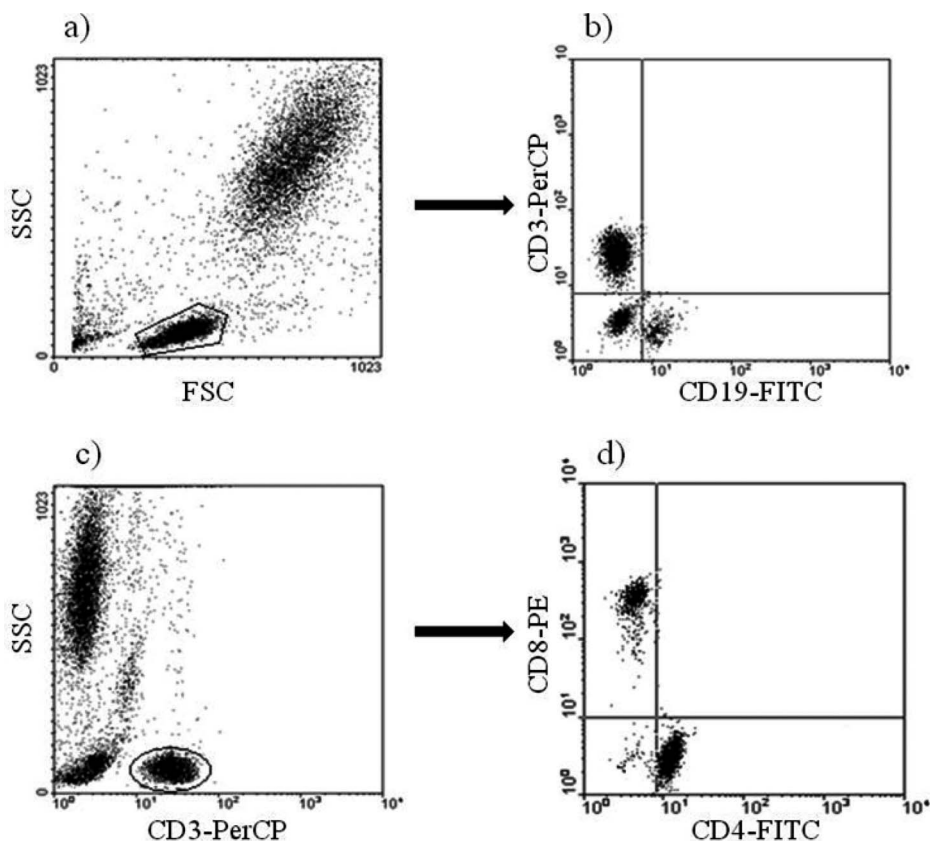


Figure 1: (a) Representative dot-plot of forward scatter (FSC) versus side scatter (SSC) showing gated lymphocytes followed by (b) dot-plot analyzing lymphocytes CD19+ (B cells) and CD3+ (T cells). (c) Representative dot-plot of CD3-PerCP versus side scatter (SSC) showing gated T cells followed by (d) dot-plot showing CD4+ and CD8+ T cells.

Statistical Analysis

Differences between groups (patients and healthy donors) were analyzed by a non-parametric Mann–Whitney U-test using SPSS version 10.00 for Windows, and significant differences were considered at $p < 0.05$.

RESULTS

In this work, double or triple labeling analyses were performed using anti-CD19FITC and anti-CD3PerCP to identify B and T lymphocytes, or anti-CD3PerCP, anti-CD4FITC and anti-CD8PE to identify T cells subsets (CD4 and CD8). The percentages of B lymphocytes were significantly lower ($p = 0.02$) in pulmonary TB patients ($n = 17$, median 10%; range: 1–14%) than in healthy controls ($n = 17$, median 12%; range: 10–21%) (Fig. 2). In contrast,

percentages of T lymphocytes (CD3+) were similar in pulmonary TB patients (median 65%; range: 20 to 79%) to those of healthy controls (median 72%; range: 51–80%) ($p = 0.12$). No significant differences in percentages of CD4+ T ($p = 0.19$) or CD8+ T ($p = 0.85$) cells between pulmonary TB patients and healthy controls were observed, neither between absolute values of CD4 ($p = 0.06$) nor CD8 cells ($p = 0.15$) (Fig. 2). Analysis of the total numbers of cells only showed significant difference in B lymphocytes between TB patients and healthy subjects ($p = 0.005$) see Figure 2.

DISCUSSION

A small number of studies have been performed to find phenotypic markers on peripheral blood that help diagnose tuberculosis. The main contribution of this research is the phenotypic characterization of peripheral blood B and T lymphocytes in patients with pulmonary tuberculosis. According to our results, the only significant difference between healthy donor and TB patient groups was in the percentage and absolute number of B lymphocytes. Pulmonary tuberculosis patients had a lower number of peripheral blood B lymphocytes than healthy donors

It is well known that CD4+ and CD8+ T lymphocyte subsets, particularly CD4+, play a very important role in the immunity against *M. tuberculosis*. CD4+ T cells are critical in the control of this infection, as has been demonstrated in animal models deficient in CD4+ T cells (Rahemtulla et al., 1991), and in HIV+ individuals latently infected with *M. tuberculosis* (Aaron et al., 2004; Flynn, 2004). There are reports that show decreased percentages of CD4 T cells, but this depends on the severity of the tuberculosis infection (Barcelos et al., 2006; Carvalho et al., 2002; Deveci et al., 2006; Rodrigues et al., 2002). In contrast, unchanged peripheral blood CD4+ T cell counts from patients with tuberculosis as compared with controls have been described (Shijubo et al., 1992; Yildiz et al., 2001).

Similarly, we did not detect any significant change in CD4+ T cells in patients with TB as compared with healthy donors. The recently described cross-priming mechanism of CD8+ T cells in human tuberculosis by mycobacterium-induced apoptotic vesicles represents a very important means of CD8+ T cells activation in *M. tuberculosis* infections (Winau et al., 2006). In this study, the percentage and the absolute counts of CD8+ T cells from patients were similar to those from healthy donors. Comparable results have been reported by other researchers (Barcelos et al., 2006; Deveci et al., 2006; Uppal et al., 2004; Yildiz et al., 2001).

The group of healthy controls was characterized by a large PPD reactivity (10 ± 4.0 mm) due to its history of BCG in agreement with the results described by Al Zahrani et al. (2000), who also reported on a significant

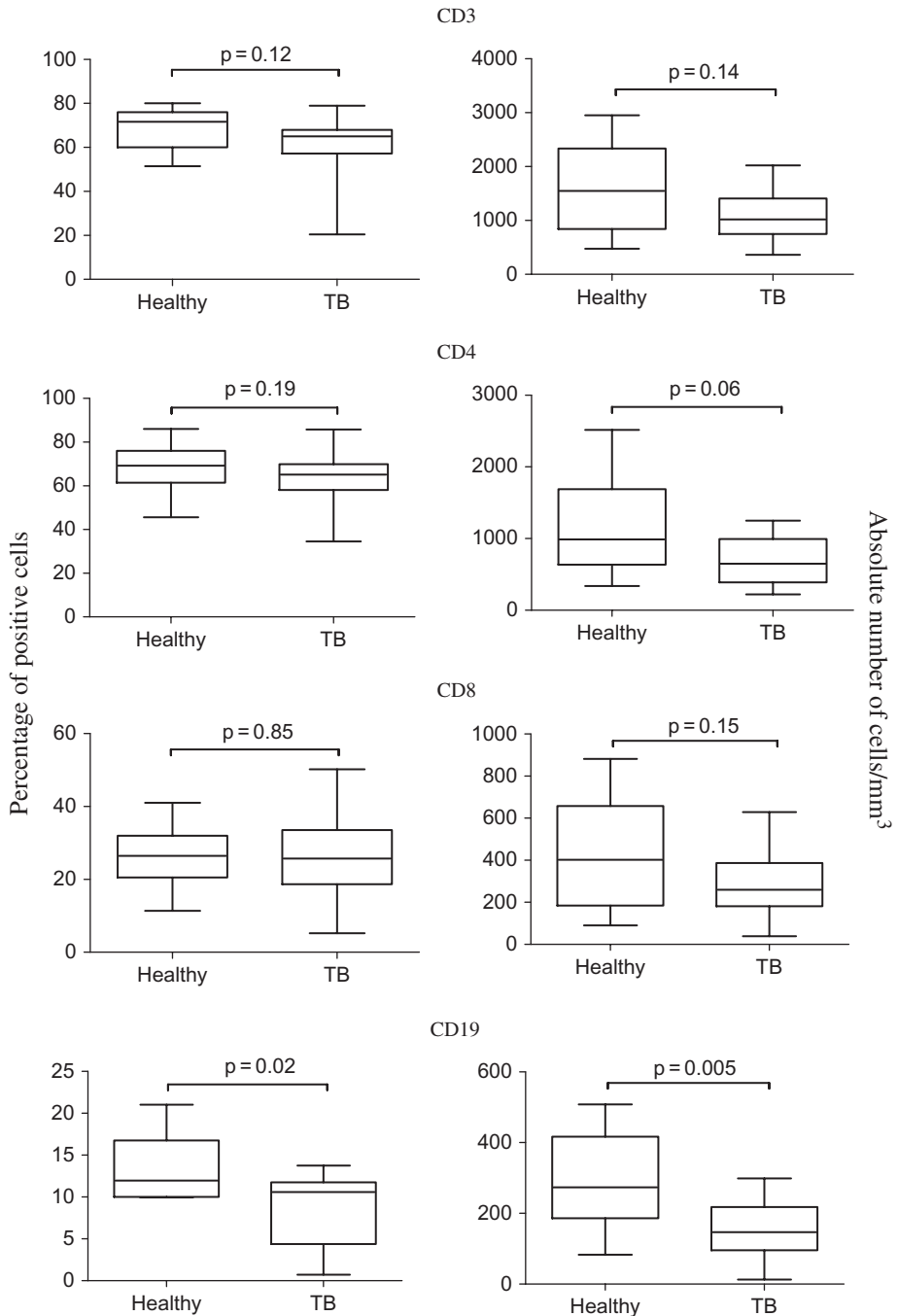


Figure 2: Percentages and absolute number/mm³ of peripheral blood CD3+ lymphocytes (T cells) and T cells expressing CD4+ or CD8+; and percentages and absolute numbers/mm³ of peripheral blood CD19+ lymphocytes (B cells). The boxes represent the median, 25/75 percentile, and extreme values. Lines showed the P-values calculated by the Mann-Whitney U-test.

percentage of TB patients with a negative or weak response to PPD, a result that was defined as anergy, coinciding with our observations.

As mentioned above, the major finding of this study was the significant reduction in the percentage and total number of B cells (CD19+) in patients with pulmonary TB compared with healthy donors. Similar results have been described by others (Barcelos et al., 2006; Corominas et al., 2004; Dubaniewicz et al., 2004; Jacobsen et al., 2006; Onwubalili et al., 1987). The role of B cells during *M. tuberculosis* infections is not totally clear. Some authors suggest that B cells participate in the control of mycobacterial infection by producing natural antibodies, but others report no differences between B cell-deficient mice and wild-type mice (Johnson et al., 1997). However, other studies suggest an important participation of B cells, acting as antigen-presenting cells and in granuloma formation (Bosio et al., 2000; Vordermeier et al., 1996). In a mouse model of chronic TB, B cells did not contribute to the natural course of chronic TB (Turner et al., 2001). However, Bosio et al. (2000) demonstrated that B cells participate in the progression of murine infection with virulent mycobacteria.

B and T cell percentage determinations are not routine tests in diagnostic laboratories. However, considering that the use of flow cytometry is increasing everyday and it is also available in developing countries such as Mexico, both in research and diagnostic laboratories, for the routine evaluation of T lymphocyte concentrations and T cell subpopulations such as CD4 and CD8 in, for instance, AIDS patients, we believe that determining B and T cell percentages by FACS analysis is an easy and useful determination that could contribute to a better management of TB patients.

Flow cytometry analysis of control and TB patient groups showed a statistically significant difference in the percentage of peripheral B-lymphocytes between control and TB patient groups. Based on this observation, our findings support the proposal that, together with clinical manifestations of pulmonary tuberculosis, a low B cell count could be used as an additional marker for pulmonary tuberculosis.

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Declaration of Interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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