

Lymphocyte Subgroups in Different Forms of Tuberculosis

Farklı Tüberküloz Formlarında Lenfosit Alt Grupları

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ABSTRACT

Objective: The balance between the bacillus and host defense mechanisms seems to be important for manifestations of tuberculosis infection. Not only subtypes of T-lymphocytes but their activation status also is important in the immunity of tuberculosis infection. The aim of this study is to address the relationship between T-lymphocyte subtypes, activation and natural killer cells with different clinical forms of mycobacterium tuberculosis infection.

Material and Method: Twenty-one active pulmonary tuberculosis, 10 inactive pulmonary tuberculosis, 10 tuberculous pleurisy patients and 18 healthy subjects were involved in the study. Immunophenotyping was carried out in peripheral blood for T cell subtypes, natural killer cells and B cells by flow-cytometry, and HLA-DR and CD25 positivity were studied to determine T-lymphocyte activation.

Results: The CD4+ T lymphocyte rates was found significantly lower in bilateral active pulmonary tuberculosis compared to healthy controls. T-lymphocyte activation was found to be increased in tuberculous pleurisy cases. The CD3/CD25+ T lymphocyte rate was significantly higher in tuberculous pleurisy cases compared both to healthy controls and inactive pulmonary tuberculosis cases.

Conclusion: CD4+ T-lymphocyte rate may be decreased in pulmonary tuberculosis patients with bilateral pulmonary infiltration. Prominent T-lymphocyte activation in tuberculous pleurisy might be a result of a hypersensitivity reaction against the bacillus. (*Tur Toraks Der 2012; 13: 1-5*)

Key words: Tuberculosis, T-lymphocyte subtypes, tuberculous pleurisy

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ÖZET

Amaç: Tüberküloz enfeksiyonunun ortaya çıkan bulgularında basil ile konakçının savunma mekanizmaları arasındaki denge önemlidir. Tüberküloz enfeksiyonunun immünesinde T lenfositlerin alt grupları kadar aktivasyon durumları da önemlidir. Bu çalışmanın amacı Mikobakterium tüberkülozis enfeksiyonunun farklı klinik formları ile T lenfosit alt grupları, aktivasyonu ve natural killer hücreler arasındaki ilişkinin araştırılmasıdır.

Gereç ve Yöntem: Yirmi bir aktif pulmoner tüberküloz, 10 inaktif pulmoner tüberküloz, 10 tüberküloz plörezi hastası ve 18 sağlıklı kontrol olgusu çalışmaya dahil edildi. Periferik kanda immün fenotiplendirme yapılarak T hücre subgrupları, natural killer hücreler ve B hücreler için flowsitometri ve T lenfosit aktivasyonunun gösterilmesi için HLA-DR ve CD25 pozitifliği çalışıldı.

Bulgular: Bilateral aktif pulmoner tüberkülozlu hastalarda kontrol grubuna göre CD4+ T lenfosit oranları anlamlı olarak düşük bulundu. Tüberküloz plörezi hastalarda T lenfosit aktivasyonunun artmış olduğu saptandı. CD3/CD25+ T lenfosit yüzdesi tüberküloz plörezi hastalarda hem sağlıklı kontrollere hem de inaktif pulmoner tüberkülozlu olgulara göre istatistiksel anlamlı olarak artmış bulundu.

Sonuç: İki taraflı parankimal infiltrasyonu olan pulmoner tüberkülozlu hastalarda CD4 T lenfosit yüzdeleri düşebilmektedir. Tüberküloz plörezi hastalarda belirgin T lenfosit aktivasyonunun gösterilmesi basile karşı hipersensitivite reaksiyonunun sonucu olabilir. (*Tur Toraks Der 2012; 13: 1-5*)

Anahtar sözcükler: Tüberküloz, T lenfosit subgrupları, tüberküloz plörezi

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INTRODUCTION

Despite the development of antituberculous drugs which have been available for more than 50 years, tuberculosis is one of the leading infectious diseases in adults, and *M. tuberculosis* infection is considered a great public health problem [1,2]. One third of the world's population is infected with *M. tuberculosis*. In Turkey, the incidence rate of tuberculosis has been reported as 26/100.000 [3].

Although tuberculosis may involve any organ of the body, it usually affects the lungs. The most common route of infection is inhalation of droplets carrying *M. tuberculosis*. This results in a local lung immune response that generally contains the infection [4]. The different manifestations of infection with *M. tuberculosis* reflect the balance between the bacillus and host defense mechanisms, in which the cellular immune response seems to be essential for preventing develop-

ment of disease. The mechanisms of protective immunity against *M. tuberculosis* in humans have not been fully clarified [5,6]. Cell-mediated immunity (CMI), especially CD4+ T lymphocyte function, is crucial in acquired immunity against the mycobacteria in tuberculosis [7]. Previous studies confirmed changes in T cells in tuberculosis patients with a decrease in CD4+ T cells and either a decrease or increase in CD8+ T cells in peripheral blood [8-10]. Together with the subtypes of T lymphocytes, their activation status also is important in the immunity of tuberculosis infection as reported by an increase in CD25 cells in patients with rapid resolution of pulmonary tuberculosis [11]. The importance of innate immunity, as detected by natural killer (NK) cells in the host defense against tuberculosis infection, has also been implicated by showing lower levels of NK cells in severely ill patients [12].

In the current study, we aimed to address the relationship of T lymphocyte subtypes, T lymphocyte activation and NK cells with different clinical forms of mycobacterium tuberculosis infection in non-HIV infected patients.

MATERIAL and METHOD

Study Population

This is a case series study including fifty-nine cases who were admitted to the Department of Chest Diseases, Eskişehir Osmangazi University Medical Faculty. None of the cases were HIV-infected and those who had any malignancy or were under immunosuppressive therapy were excluded. The study protocol was approved by the ethics committee of Eskişehir Osmangazi University and both verbal and written informed consent were obtained from each participant prior to the study.

Twenty one of the patients had active pulmonary tuberculosis. The diagnosis of active pulmonary tuberculosis patients was based either on a positive sputum smear result for acid fast bacilli, confirmed by a positive culture of *M. tuberculosis* in patients with characteristic clinical and radiological features of the infection (smear-positive pulmonary TB), or on a culture positivity for *M. tuberculosis* in patients having at least 2 sputum specimens negative for acid-fast bacilli (smear-negative pulmonary TB). Ten of the patients had inactive pulmonary tuberculosis. The diagnosis of inactive pulmonary tuberculosis was based on a positive history of tuberculosis therapy with sequel lesions on chest radiography, and negative sputum smears and cultures for *M. tuberculosis*. Ten of the patients had the diagnosis of tuberculosis pleurisy diagnosed by a histopathologic examination of pleural biopsy revealing granulomatous inflammation with caseification necrosis. The general condition of all patients was good and none of them had cachexia. Eighteen healthy control subjects were involved in the study.

Laboratory examinations (complete blood count, T lymphocyte subgroup markers) were carried out by a

technician blind to the clinical data and according to current standards. Tuberculin skin test (TST) was done for all patients. TST was performed by trained personnel following standard procedures. In brief, 0.1 mL (2 TU) of purified protein derivate was injected intradermally on the volar side of the forearm and the transverse diameter of the induration was read 72 h later. A diameter ≥ 10 mm was considered positive.

Flow Cytometry Immunophenotyping:

Peripheral venous blood samples (5 ml) were collected into tubes with EDTA from all subjects and were subjected to flow cytometry within two hours. Afterwards, 20 μ L of fluorochrome-conjugated monoclonal antibody were added to 100 μ L of whole blood in a tube, vortexed gently and incubated for 20 minutes in the dark at room temperature. Then 2 mL of 1x Becton Dickinson (BD) FACS lysing solution was added, again vortexed gently and incubated for 10 minutes in the dark at room temperature. The materials were centrifuged at 300 x g for 5 minutes, washed with 2 mL of BD cell-WASH and centrifuged again at 200 x g for 5 minutes and the supernatant was removed. After 0.5 mL of BD cellFIX solution was added the remaining cells were analysed in BD FACS Calibur in the cellquest programme.

Immunophenotyping was done in peripheral whole blood T cells and T cell subtypes (CD3+CD4+, CD3+CD25+, CD3+CD8+, CD3+antiHLA-DR+), natural killer (NK) cells (CD3-, CD16+/CD56+) and B cells (CD3-, CD19+) from patients and controls by using the following monoclonal antibodies: CD3FITC/CD4PE, CD3FITC/CD8PE, CD3FITC/16+56PE, CD3FITC/CD19PE, CD25FITC, anti HLA-DR FITC.

In the analysis of cytofluorimetric data (FACS Calibur, Becton-Dickinson) the cellQuest program was used to optimize gating of lymphocytes in order to provide an objective means of excluding both debris and erythrocytes.

Statistical Analysis

Results are expressed as median (range). Multiple group comparisons were made using Kruskal Wallis. The Mann-Whitney U test was used for two-sided comparisons. A p value less than 0.05 was considered as statistically significant. The personal computer program SPSS for Windows (13.0) was used for all calculations.

RESULTS

As shown in Table 1, the ages of the four study populations were similar and the male/female ratios were evenly distributed in the four groups ($p=0.347$ and $p=0.474$, respectively). In the comparison of clinical data between the four groups, the sedimentation rate was significantly higher in active TB cases compared to inactive TB and control cases ($p=0.02$, $p<0.001$; respectively) and was significantly higher in TB pleurisy cases compared to healthy controls ($p=0.001$). TST measurement

Table 1. Characteristics of study groups

	Active pulmonary TB (n=21)	Inactive TB (n=10)	TB pleurisy (n=10)	Healthy controls (n=18)	p
Age (years)	44 (40)	50 (17)	46 (47)	41 (39)	0.347
Male/Female (n)	12/9	7/3	4/6	8/10	0.474
Sedimentation rate (mm/hr)	80 (101)	31.5 (62)	56.5 (83)	21.0 (14.0)	<0.001*
TST (mm)	15 (20)	12 (25)	11 (20)	5 (15)	0.022**

Results are presented as median (range). $p < 0.05$ is considered significant, TB: tuberculosis; mm: milimeter; hr: hour, *: Sedimentation rate was significantly higher in active pulmonary TB compared to inactive pulmonary TB cases ($p = 0.02$) and healthy controls ($p < 0.001$); and was significantly higher in TB pleurisy cases compared to healthy controls ($p = 0.001$), **: TST measurement was significantly higher in active pulmonary TB cases compared to healthy controls ($p = 0.035$)

was significantly higher in active pulmonary TB cases compared to healthy controls ($p = 0.035$).

Median percentage of CD3+, CD3+/CD4+, CD3+/CD8+, CD3-/CD19+, CD16/56, CD3+/CD25+ and CD3+/HLADR+ cells were 68.7 (41.8), 41.4 (62.1), 26.4 (43.2), 8.5 (21.6), 15.3 (48), 6.7 (70.5) and 10.7 (51.5) respectively. The CD3+/CD25+ T cell percentage was significantly different between the four groups. In multiple comparisons, percentages of CD3+/CD25+ T cells were higher in TB pleurisy cases than in inactive TB patients and healthy controls ($p = 0.023$ and $p = 0.024$; respectively) (Figure 1).

Active pulmonary TB cases were classified according to their radiological involvement. Of the 21 pulmonary TB cases, 12 had unilateral and 9 had bilateral involvement. The percentage of CD3+/CD4+ lymphocytes was found significantly lower in bilateral active TB cases compared to the control group (28.0 (41.0)% versus 45.4 (48.4)%; $p = 0.003$), whereas no significant difference was found between unilateral pulmonary TB cases and healthy controls (Figure 2). There was no significant difference in respect to other cells regarding involvement.

DISCUSSION

Mycobacterium tuberculosis is an intracellular pathogen capable of persisting and replicating within the cells of the mononuclear phagocyte system. Local innate immunity mediated primarily by alveolar macrophages usually fails to control the slowly replicating bacilli. Adaptive immunity develops upon exposition of the immune system to mycobacterial antigens. Yet the adaptive immunity mediated by T cells does not eradicate the infection and ongoing protective immunity is required for the control of persistent bacilli [13,14].

Ninety percent of individuals who are infected with *M. tuberculosis* never develop clinical disease. Bacterial and host factors that adversely affect these two arms of the immune system are the contributing factors to latent tuberculosis infection (LTBI) and active disease [12].

Active tuberculosis (TB) is characterized by a profound and prolonged suppression of *Mycobacterium tuberculosis* (MTB)-specific T cell responses, as evidenced

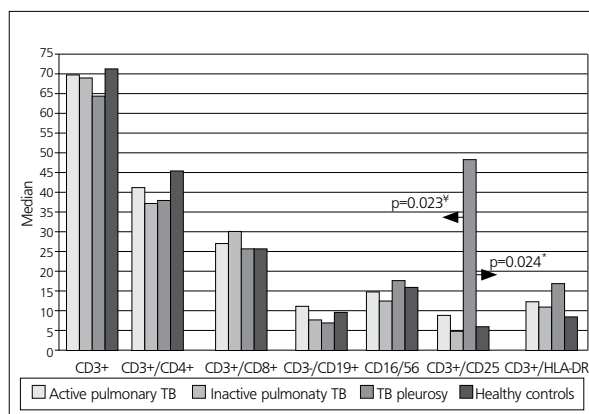


Figure 1. Lymphocyte subgroups in patients with active pulmonary tuberculosis, inactive pulmonary tuberculosis, tuberculous pleurisy and in healthy controls

*In patients with tuberculous pleurisy, the CD3/CD25+rate is significantly higher compared to healthy controls ($p = 0.024$)

†In patients with tuberculous pleurisy, the CD3/CD25+rate is significantly higher compared to inactive pulmonary TB cases ($p = 0.023$)

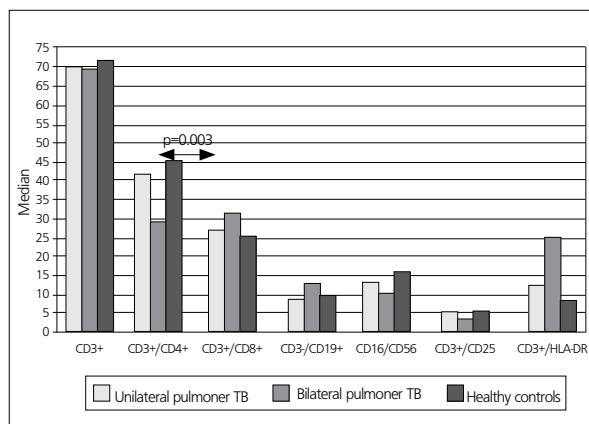


Figure 2. Lymphocyte subgroups in patients with active pulmonary tuberculosis according to disease severity. In patients with bilateral active pulmonary tuberculosis, the CD3/CD4+ percentage is significantly lower compared to healthy controls ($p = 0.003$)

by decreased production of the cytokines interleukin (IL)-2 and interferon (IFN)- γ [15]. *M. tuberculosis* is also known to interfere with antigen presentation by MHC class II molecules, which is important for priming CD4 T cells. Such interference could lead to resistance of *M. tuberculosis* to acquired immunity in the host, and

hence may contribute to bacterial persistence, a characteristic feature of this organism. *M. tuberculosis* products suggested to inhibit macrophage presentation of MHC class II molecules include lipoarabinomannan, 25 kDa glycoprotein and 19 kDa lipoprotein [16].

CD4+ T-cells play the central role in protective immunity to *M. tuberculosis*. They secrete macrophage activating cytokines such as IFN- γ and TNF- α . It is well-known that in HIV infected patients, the CD4 cell count is important for progressive primary infection, reactivation and reinfection. Also depletion of CD4 cells experimentally in mice leads to similar findings (14). Supporting the importance of the role of CD 4 cells in tuberculosis, in the present study, the CD3+/CD4+ T cell percentage was significantly lower in bilateral active pulmonary TB cases compared to healthy controls. Previously in different studies it was shown that, in pulmonary TB patients with poor general condition, CD 4 T lymphocytes were lower compared to pulmonary [17,18] TB patients in good condition. The present study revealed that radiological involvement also has an effect on CD4 lymphocytopenia independent of the general condition of the patients.

In order to elicit the role of T lymphocytes in the pathogenesis of tuberculosis, lymphocyte subpopulations have been studied previously by different authors. An increased CD4 percentage together with decreased CD8 percentage and CD4/CD8 ratio in BAL fluid from the infected lungs of pulmonary TB patients was demonstrated [19-21]. In contrast, in these patients the CD4 percentage is decreased and CD8 percentage is increased in the peripheral blood; indicating compartmentalization of CD4 T cells in the infected lung tissues [19]. Similarly, an increased rate of CD4/CD8 in the granulomas was revealed in lung biopsies [20]. CD4 T cell percentages are further increased in BAL fluid and further decreased in peripheral blood in significantly advanced pulmonary TB cases [19]. The finding of this study concerning decreased CD4 T cells in peripheral blood in patients with bilateral active TB suggests the compartmentalization of the cells in lung tissues, although tissue biopsies were not performed in the setting of the study since it would have been an invasive procedure.

Studies concerning T cell subsets in TB pleuritis note an increased CD4/CD8 ratio in pleural fluid and increased levels of IFN- γ , reflecting T helper 1 activity [22-24]. Prabha et al. [25] further demonstrated that IFN- γ + CD4 T lymphocytes are concentrated in pleural fluid in these patients. In the present study, it was shown that although tuberculous pleurosy patients have some degree of decreased CD4+ T lymphocytes in their blood, which is statistically nonsignificant, they exert prominent T lymphocyte activation, shown by significantly increased CD3+/CD25+ T cells, probably a result of hypersensitivity reaction to the bacillus.

In the current study, no difference was found in CD3-/CD19+ cells between the four groups. In previous studies little or no contribution of B cells was reported in

the control or exacerbation of tuberculosis infection. Similarly in this study insignificance of B cells was also the case in the TB pleurisy [26-28].

NK cells are also important in the host defense against tuberculosis infection [12]. Previously, an increase in the percentage and activity of NK cells was reported in active pulmonary tuberculosis [28,29]. However, in the current study no difference was noted between the tuberculosis patients and healthy controls.

Conflict of Interest

No conflict of interest was declared by the authors.

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