CD4+ Cell Counts in Patients with Different Clinical Manifestations of Tuberculosis

Setareh Davoudi[1], Mehrnaz Rasoolinegad[1], Masoud Younesian[2], Mahboubeh Hajiabdolbaghi[1], Abdolreza Soudbakhsh[1], Sirous Jafari[1], Hamid EmadiKouchak[1], Morteza Mehrpouya[1] and Hossein Lotfi[1]

[1]Department of Infectious Diseases, Imam Khomeini Hospital, Tehran University of Medical Sciences; [2]School of Public Health, Tehran University of Medical Sciences; Tehran, Iran

Tuberculosis is the prototype of infections that require a cellular immune response for their control. It has been shown that CD4+ T-lymphocytes are most important in the protective response against *Mycobacterium tuberculosis*. CD8+ T-lymphocytes are also important for effective T-cell immune response. This study compares CD4+ and CD8+ baseline values in patients with different manifestations of tuberculosis. CD4+ and CD8+ in three groups of patients with tuberculosis (pulmonary, lymphadenitis, meningitis/milliary involvement) and a group of healthy volunteers were enumerated using flowcytometry. Twenty-six patients with pulmonary tuberculosis, 10 with adenitis, 16 with meningitis or milliary tuberculosis and 16 healthy volunteers entered the study. Mean CD4 in meningitis/ milliary group was significantly lower than all other groups ($p<0.05$). Mean CD4 counts of patients with pulmonary tuberculosis was also significantly lower than control group ($p=0.01$). Mean CD8 in meningitis/milliary group was significantly lower than control group ($p=0.02$). No relation was found between results of TSTs and CD4 values in three groups. CD4 depletion is an expectable phenomenon in patients with tuberculosis. This study shows that patients with more severe form of disease had the lowest number of both CD4 and CD8 cells which can be a sign of suppressed cellular immunity in these patients.

Key-Words: Tuberculosis, *Mycobacterium tuberculosis*, CD4.

Tuberculosis is still one of the major causes of infectious disease related deaths. About 1.8 billion people are infected with tuberculosis bacillus. Three million die because of this disease annually [1]. Tuberculosis is the prototype of infections that require a cellular immune response for their control. It has been shown that CD4+ T-lymphocytes are most important in the protective response against *Mycobacterium tuberculosis*. In murine studies, T-cell deficiency was associated with increased susceptibility to disease [2-4]. Increased frequency and severity of tuberculosis in HIV positive patients is another good evidence of importance of CD4+ T-cells in control of this infection. CD8+ T-lymphocytes are also important for effective T-cell immune response [5]. They are capable of secreting cytokines such as IFN-γ and IL-4 and thus may play a role in regulating the balance of Th1 and Th2 cells in the lungs of patients with pulmonary tuberculosis [2]. CD4+ and CD8+ T-cells share several effectors functions that mediate antimycobacterial activities and CD8+ T-cells responses should as well be stimulated to provide protective immunity [5]. The aim of this study is to determine and compare CD4+ and CD8+ baseline values in patients with different manifestations of tuberculosis and a healthy control group.

Material and Methods

This study was performed in Imam Khomeini Hospital, Tehran, Iran, during two years period (2003-2005). Three groups of patients and one group of healthy volunteers were enrolled in the study. Patients consisted of those with pulmonary tuberculosis, tuberculosis lymphadenitis and milliary or meningitis. Patients in each group considered eligible if they meet the following criteria:

- Pulmonary tuberculosis: patients with two positive sputum smears or one positive sputum smear and chest radiography compatible with tuberculosis.
- Tuberculosis lymphadenitis: peripheral lymphadenopathy with positive tissue culture or pathologic findings suggestive of tuberculosis.
- Tuberculosis meningitis: cases with chronic meningitis plus one of the following: A) Positive CSF smear, culture or PCR; B) CSF analysis compatible with meningitis plus increased CSF adenosine deaminase level in whom other causes of chronic meningitis had been ruled out and antituberculous drugs were initiated by an infectious disease specialist.
- Milliary tuberculosis: involvement of two or more non contiguous organs with pathologic or microbiologic evidence of tuberculosis in at least one of them and/or milliary pattern in chest radiography.
- Members of control group were chosen from healthy volunteers with no history of tuberculosis and negative tuberculin skin test (TST) results. Those with HIV infection or any other immunodeficiency disorders, renal failure, diabetes mellitus and those receiving corticosteroid or any other immunosuppressive drugs were excluded from the study. Two millilitre of whole blood with EDTA (as anticoagulant) was obtained. CD4+ and CD8+ were enumerated by flowcytometry.

Data were analyzed by SPSS 11.5. Mean values calculated and were compared using one way ANOVA test. Significant differences were evaluated between groups by Post Hoc Tukey test. For comparison of PPD skin test results between

Received on 20 August 2008; revised 14 November 2008.

Address for correspondence: Dr. Setareh Davoudi.Assistant Professor of Infectious Disease, Imam Khomeini Hospital, Keshavarz Boulevard, Tehran-IRAN. Zip code: 1419733141. Phone/Fax: +98 2166581598. E-mail: davoudis@tums.ac.ir.

The Brazilian Journal of Infectious Diseases 2008;12(6):483-486.

different groups, chi-square was used. Student t test was used for comparison of mean CD4 count between PPD positive and negative.

Results

Sixty-eight subjects entered the study: 26 with pulmonary tuberculosis, 10 with adenitis, 16 with meningitis or milliary tuberculosis and 16 healthy volunteers. There were no significant difference between mean age (p=0.06) and sex distribution (p=0.68) of these four groups (Table 1). The mean number of CD4+ T-cells, CD8+ T-cells and CD4/CD8 ratio of each group is shown in Table 2. There was a significant difference between mean CD4 values in four groups. Post Hoc Tukey test showed that mean CD4 in meningitis/milliary group was significantly lower than all other groups including: pulmonary (p=0.04), adenitis (p=0.01) and control group (p<0.001). Mean CD4 counts of patients with pulmonary tuberculosis was also significantly lower than control group (p=0.01). There was no significant difference in mean CD4 between neither pulmonary and adenitis (p=0.74) nor adenitis and control group (p=0.46). Eleven patients, 5 (19.2%) in pulmonary group and 6 (37.5%) with meningitis/milliary involvement had CD4 counts less than 300. A significant difference was observed between mean CD8 values in different groups. Further analysis showed that only mean CD8 in meningitis/milliary group was significantly lower than control group (p=0.02). CD4/CD8 ratio in different groups was not significantly different. Tuberculin skin test had been performed for 63 persons. 61.9% of patients in pulmonary tuberculosis group had TST ≥10mm (which was considered as positive), whereas most patients in meningitis/milliary (68.8%) and adenitis (60%) groups had TST<10mm (p=0.05). All healthy controls had negative TST (TST<10mm) (Table 3).

No relation between results of TSTs and CD4 values in three groups was found (Table 4). Interestingly patients with TST<10mm in adenitis group had higher CD4 counts than those with TST ≥10mm in same group.

Discussion

One-third of world population is infected with *Mycobacterium.tuberculosis*, but only 5% to 10% develop active disease. Even the active disease can present as different clinical forms with highly variable localization and severity. The different manifestations of infection reflect the balance between the bacillus and host defense mechanism, in which the quality of host defense play detrimental role [6-8]. *Mycobacterium.tuberculosis* is a classic example of a pathogen for which the protective response relies on cell mediated immunity. Both CD4 + and CD8+ T-cells are important for successful immunity to tuberculosis. They have many effector functions such as cytolysis and release of potent antimycobacterial cytokines like IFN-γ and TNF-α [1,5]. In this study CD4+ and CD8+ cell counts were compared in three different forms of tuberculosis. Pulmonary involvement as a classic form of tuberculosis, peripheral lymphadenitis as the most common and a benign form of extrapulmonary tuberculosis and a group of meningitis or milliary representing severe and/or disseminated form of disease. The results showed that patients with pulmonary tuberculosis had lower CD4 counts than control group. CD4 lymphocytopenia had been previously shown in both smear positive [3,9-11] and smear negative [9] pulmonary tuberculosis. Another study revealed increased CD4+ T lymphocytes in BAL fluid of patients with less advanced non-cavitary pulmonary tuberculosis and significant enhancement in percentage of CD8+ cells in severe cavitary forms [7]. In present study patients in meningitis/milliary group had the lowest number of CD4 counts which was even significantly lower than other patients including pulmonary and adenitis. In another study the frequency of extrapulmonary involvement including milliary was higher among patients with lower CD4 [12]. In a group of patients with mixed type of tuberculosis low CD4 count was observed in patients without separating pulmonary from extrapulmonary forms [13], whereas in one study no significant difference was reported between mean CD4 count of patients with pulmonary and extrapulmonary tuberculosis, again without mentioning the site of extrapulmonary involvement. The authors concluded that patients with lower CD4 counts had more severe disease and poorer condition as it was shown before [14,15]. Several hypotheses have been postulated for diminished number of CD4+ cells in tuberculosis. Lymphocytes homing in affected tissue rather than circulation, increased apoptosis level [3] or impaired thymus function [16] are some examples.

The results of our study also showed that patients in meningitis/milliary group had the lowest number of CD8 cells which was significantly different from control group. The results of other researches were somehow conflicting. Some studies showed normal CD8 values in patients [11,13], some increased [9] and some decreased values [3]. Recent studies suggest that CD8 T-cells have distinctive role in immunity against *Mycobacterium tuberculosis*, for example they preferentially use granule exocytosis pathway and recognize heavily infected cells [5], ingest macrophages that have engulfed mycobacteria [7], secrete cytokines and lyse infected cells [6].

It is rationally assumed that as cutaneous response to PPD is a result of CMI activation and directly related to CD4 numbers, patients with lower CD4 counts should have poorer response [12]. But this study did not find any relation between CD4 counts and results of TST like some other studies [13]. Again lymphocyte homing is a probable justification, but the finding that patients with TST<10mm in adenitis group had higher CD4 counts than those with TST ≥10mm in same group does not correlate with this assumption either. It is worth mentioning that many factors including technical problems and underlying host conditions like malnutrition or simultaneous viral infections can easily affect the result of TST, so we can not rule out the importance of CD4 cells as main soldiers of CMI only by considering TST results.

Table 1. Age and sex distribution of study groups.

Study groups	No.of cases	Mean age± SD	Gender	
			Male (%)	Female (%)
Pulmonary	26	47.12±20.58	15 (57.7)	11 (42.3)
Adenitis	10	34.5±13.28	4 (40)	6 (60)
Meningitis/Milliary	16	38.75±27.64	10 (62.5)	6 (37.5)
Healthy controls	16	32.81±49.8	8 (50)	8 (50)
Total	68	40.22±18.86	37 (54.4)	31 (45.6)

Table 2. Mean values of CD4, CD8 T-lymphocytes and CD4/CD8 ratio in different study groups.

Study groups	CD4/CD8 ratio (Mean ± SD)	CD8+ cells (Mean ± SD)	CD4 + cells (Mean ± SD)
Pulmonary	1.32±0.67	559.96±379.37	695.42±409.77
Adenitis	1.58±0.84	556.2±267.42	831.4±522.87
Meningitis/Milliary	1.33±0.62	337.38±185.69	384.94±156.66
Healthy controls	1.75±0.64	636.38±172.59	1045.94±316.46
P value	0.19	0.02	<0.001

Table 3. TST results in study groups.

Study groups	TST results	
	<10 mm	≥10mm
Pulmonary No. (%)	8 (38.1)	13 (61.9)
Adenitis No. (%)	6 (60)	4 (40)
Meningitis/Milliary No. (%)	11 (68.8)	5 (31.3)
Healthy controls No. (%)	16 (100)	0

Table 4. Mean CD4+ values in cases according to TST results.

	CD4+ cells values(Mean ± SD)		
TST results	Pulmonary	Meningitis/milliary	Adenitis
<10mm	685.13±541.62	383.91±174.62	963.67±613.42
≥10mm	755.77±386.87	387.2±125.67	633±324.91
P value	0.27	0.24	0.20

According to our results patients with more severe form of disease had the lowest number of both CD4 and CD8 cells which can be a sign of suppressed cellular immunity in these patients. We can not conclude by this study that whether this depletion was caused by tuberculosis or predisposed patients to tuberculosis. But some other studies which have shown that this is a reversible phenomenon which returns to normal with successful antituberculous therapy, suspected tuberculosis of causing lymphocytopenia [3,11,13,14,17]. However, during this period, degree of immunesuppression may be so severe that the patients fall in the category of CD4 lymphocytopenia [12,18]. So CD4 depletion should always be in mind in approach to patients with tuberculosis and before any decision is made regarding this finding a response to antituberculous treatment should be evaluated.

This study again highlights the importance of cellular immunity, conducted by T-lymphocytes, in the outcome of tuberculosis. Although the role of effective antimycobacterial drugs as a major adjunctive can not be forgotten, the emergence of multidrug resistance mycobacterium poses new problem in controlling this reemerging disease [19]. The need for modalities such as vaccines and immunotherapy is felt more than ever. The more we learn about disease pathogenesis, the faster we can move toward success in the battle between human and *Mycobacterium tuberculosis*.

Acknowledgement

The authors would like to thank the immunology, asthma and allergy research center for their collaboration in performing flowcytometry and Dr. Makarem for his help in data analysis.This research was supported with a grant from Tehran university of Medical Sciences.

References

1. World Health Organization. The world Health Report: Making a difference; **1999** p:25.
2. Raja A. Immunology of Tuberculosis. Indian J Med Res **2004**;120:213-32.
3. Rodrigues D.S., Medeiro E.A.S., Weckx L.Y., et al. Immunophenotypic characterization of peripheral T lymphocytes in *Mycobacterium*.tuberculosis infection and disease. Clin Exp Immunol **2002**;128:149-54.
4. Scanga C.A., Mohan V.P., Yu K., et al. Depletion of CD4+ T cells causes reactivation of murine persistent tuberculosis despite continued expression of interferon-γ and nitric oxide synthase. J Exp Med **2000**;.192:347-58.
5. Lewinsohn D.A., Heinzel A., Garadner J.M., et al. *Mycobacterium tuberculosis* specific CD8 T cell preferentially recognize heavily infected cells. Am J Respir Crit Care Med **2003**;168:1346-52.
6. van Crevel R., Ottenhoff T.H.M., van der Meer J.W.M. Innate immunity to *Mycobacterium tuberculosis*. Clin Microbiol Rev **2002**;15:294-309.
7. Mazzarella G., Bianco A., Perna D., et al. T lymphocyte phenotypic profile in lung segments affected by cavitary and non-cavitory tuberculosis. Clin Exp Immunol **2003**;132:283-8.

8. Flynn J.L., Chan J. Immune evasion by *Mycobacterium tuberculosis*: living with the enemy. Current Opinion in Immunology **2003**;15:450-5.
9. Singhol M., Banavalikar J.N. Peripheral blood T lymphocyte subpopulations in patients with tuberculosis and the effect of chemotherapy. Tubercle **1989**;70:171-8.
10. Villacian J.C., Tan G.B., Teo L.F., Paton N.I. The effect of infection with *Mycobacterium tuberculosis* on T-cell activation and proliferation in patient with and without HIV co-infection. J Infect **2005**;51:408-12.
11. Uppal S.S., Tewari S.C., Verma S., Dhot P.S. Comparison of CD4 and CD8 lymphocyte counts in HIV-negative pulmonary TB patients with those in normal blood donors and the effect of antitubercular treatment: Hospital-based flow cytometric study. Cytometry B Clin Cytom **2004**;61:20-6.
12. Kony S.J., Hane A.A., Larouze B., et al. Tuberculosis-associated severe CD4+ T-Lymphocytopenia in HIV-seronegative patients from Dakar. J Infect **2000**;41:167-71.
13. Onwubalili J.K., Edwards A.J., Palmer L. T4 Iymphopenia in human tuberculosis. Tubercle **1987**;68:195-200.
14. Jones B.E., Oo M.M., Taikwel E.K., et al. CD4+ cell count in human immunodeficiency virus-negative patients with tuberculosis. Clin Infect Dis **1997**;24:988-91.
15. Pilheu J.A., De Salvo M.C., Gonzalez D., et al. CD+4 T-Lymphocytopenia in severe pulmonary tuberculosis whithout evidence of human immunodeficiency virus infection. Int J Tuberc Lung Dis **1997**;1:422-6.
16. Ozeki Y., Kaneda K., Fujiwara N., et al. *In vivo* induction of apoptosis in the thymus by administration of mycobacterial cord factor. Infect immun **1997**;65:1793-9.
17. Turett G.S., Telzac E.E. Normalization of CD+ T lymphocyte depletion in patients without HIV infection treated for tuberculosis. Chest **1994**;105:1335-7.
18. CDC. Unexplained CD4 T lymphocytopenia in persons without evident HIV infection MMWR **1992**;41:541-5.
19. World Health Organization. The world Health Report: A safer future **2007**:52-4.