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Full Length Research Paper

Pulmonary tuberculosis associated with increased number and percentage of natural killer and B cells in the peripheral blood

Adel Almogren

Department of Pathology, College of Medicine, King Saud University, P. O. Box 2925, Riyadh 11461, Kingdom of Saudi Arabia. E-mail: almogren@ksu.edu.sa. Tel: 00-966-1-4671843. Fax: 00-966-1-4671925.

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Host defense against *Mycobacterium tuberculosis* (MTB) is essentially a cell mediated immune response. The aim of this study is to assess immune abnormalities in the peripheral blood lymphocyte subsets in patients with pulmonary tuberculosis. Flowcytometry data for peripheral blood lymphocyte subsets in ten patients (mean age of 27 ± 6 years) with pulmonary tuberculosis were compared with similar data from 25 normal healthy individuals (mean age 24 ± 6 years) retrospectively in Immunology Unit at King Khalid University Hospital, Riyadh. The absolute numbers (523.7 ± 360.9 vs 177.1 ± 133.7, p = 0.0000) and % (28 ± 12.8% vs 9.9 ± 5.6%, p = 0.0000) of the natural killer cells and B lymphocytes (426.8 ± 452.1 vs 205.7 ± 69 p = 0.0000 and 18.2 ± 8.1% vs 11 ± 2.5%, p = 0.0000, respectively) were significantly higher in patients with PTB than the normal healthy individuals. A marked reduction in the absolute numbers (542.9 ± 350.3 vs 775.7 ± 225.4, p = 0.0250) and the percentage (30.8 ± 10.7% vs 44.01 ± 5.4%, p = 0.0000) of CD4 + cells in patients with pulmonary tuberculosis was also noted. Elevated natural killer and B cells with CD4 + lymphopenia in pulmonary tuberculosis prompt further investigations to gain a better understanding of host defense against *M. tuberculosis*.

Key words: *Mycobacterium tuberculosis,* natural killer cells, lymphocyte subsets, pulmonary tuberculosis.

INTRODUCTION

Mycobacterium tuberculosis (MTB) infection remains a major health problem in many countries of the world. Emergence of human immunodeficiency virus (HIV) infection has contributed significantly to the increase in worldwide incidence of tuberculosis (Raviglione et al., 1992). Although, one of the third of the world population is currently estimated to be infected by MTB, only 5 to 10% of these individuals develop active disease indicating immune responses controlling the infection (Glassroth, 2004; Kunst, 2006).

Cell mediated immune response is believed to be an important host response to prevent clinically evident MTB infection. CD4 positive T cells in collaboration with other T cell subsets such as CD8 positive lymphocytes have been shown to be the key players in defending the host infected with MTB (Boom et al., 2003). Presentation of MTB antigens to CD4 positive lymphocytes result in activation of this lymphocyte subset (Blythe et al., 2007) with a consequent release of interferon gamma (INFγ) that provides protection against MTB infection (Jacobsen et al., 2008). natural killer (NK) cell is an another important subset of lymphocytes that are not only capable of producing IFNγ along with other cytokines but also mediate killing of intracellular MTB (Bancroft 1993; Biron et al., 1999; Campos–Martin et al., 2004; Brill et al., 2001).

Optimal number and proportions of lymphocyte subsets are vital for induction of adaptive immunity against MTB. Alterations in T cell counts in the peripheral blood are pivotal immune abnormalities observed in patients infected with HIV (Jiang et al., 2005), thus predisposing these patients to MTB infection. The immunodeficiency state may further be aggravated by the fact that MTB has been implicated to cause CD4+ lymphopenia (Pilheu et al., 1997). Assessment of lymphocyte subsets in non-HIV infected patients with tuberculosis may therefore help in understanding the immune abnormalities associated with the condition.

Table 1. Specificity of each labeled monoclonal antibody used in the study.

S. No.	Labeled monoclonal antibody	Target cell
1.	Anti-CD3 FITC	T lymphocytes
2.	Anti-CD4 FITC	T helper lymphocytes
3.	Anti-CD8 PE	Cytotoxic T lymphocytes
4.	Anti-CD19 PE	B lymphocytes
5.	Anti-CD56+CD16 PE	Natural killer (NK) Cells
6.	Anti-HLA-DR PE	Activated lymphocytes
7.	Mouse IgG1FITC	Isotype control
8.	Mouse IgG2 PE	Isotype control

FITC = Fluorescein Isothiocyanate, PE = Phycoerythrin.

This is a retrospective analysis of flowcytometry data for peripheral blood lymphocyte subsets in patients with pulmonary tuberculosis (PTB). The aim of the study was to investigate immune abnormalities associated with MTB infection.

MATERIALS AND METHODS

Study Population

Laboratory data for peripheral blood lymphocyte subset analysis from ten patients with the diagnosis of PTB were examined retrospectively in the Immunology Unit at King Khalid University Hospital Riyadh. There were 4 female and 6 male patients with the mean age of 27 ± 6 years. Diagnosis of PTB was confirmed on clinical, radiological and microbiological evidence. Blood samples were collected prior to initiation of anti-tuberculosis therapy. None of the patients included in the study had any evidence of suffering from diabetes, HIV infection or autoimmune diseases. The study was limited by lack of access to the patient records therefore it was not possible to correlate the laboratory data with the clinical findings. Peripheral blood subset data of the patients were compared with a section of the similar data generated previously for defining the normal range of adult peripheral blood lymphocyte subsets for the Immunology laboratory at King Khalid University Hospital. This group included 8 females and 17 males with the mean age 24 ± 6 years.

Sample collection

A 5 ml sample of peripheral blood was collected from each individual using ethylenediaminetetraacetic acid (EDTA) as the anticoagulant. After the collection, the whole peripheral blood immunophenotyping was performed by flowcytometry according to the protocol of the Centre for Infectious Diseases, USA (Calvelli et al., 1993). Briefly, 100 µl aliquots of peripheral total blood collected in EDTA were added to 20 µl of relevant monoclonal antibodies (mAbs) . The labeled monoclonal antibodies used in the study against cell surface markers included anti-CD3, CD4, CD8, , CD19, CD56 + CD16 and HLA-DR. Isotypic controls included IgG1 labeled with Fluorescein Isothiocyanate (FITC) and IgG2 labeled with Phychoerythrin (PE) mouse antibodies. Table. 1 shows the specificity of the mAbs for each cell type. Following incubation with the relevant mAbs, erythrocytes were lysed using 2 ml of fluorescence-activated cell sorter (FACS) Lysing Solution (Becton Dickinson, Biosciences Pharmigen, San Diego, CA and USA). After lysing the erythrocytes cells were washed twice with 0.5 ml of phosphate-buffered saline containing 0.01% sodium azide. Cell preparations were fixed in 200 ml of FACS fix solution (10 g ⁄ l paraformaldehyde, 1% sodiumcacodylate, 6.65 g ⁄ l sodium chloride, 0.01% sodium azide). Cytofluorimetric data acquisition was performed with a Becton Dickinson FACScalibur instrument. CELLQUEST TM software (BD Bioscience, San Jose, CA, USA) provided by the manufacturer was used for data acquisition and analysis.

Statistical analysis

Analyses of the data were performed using Statistical Package for Social Sciences (SPSS) statistical software (Version 16.0). Student *t* test was applied for comparison of percentages and absolute numbers between patients and normal individuals. The difference was considered statistically significant when the p value was either equal to or less than 0.05.

RESULTS

Table 2 shows comparison of percentages of the peripheral blood lymphocytes subsets from patients with PTB and normal healthy individuals. Whereas the patients with PTB were found to have a significantly higher (p = 0.000) % of NK cells and B lymphocytes, the normal healthy individuals had a higher (p = 0.000) % of CD3 + and CD4 + lymphocytes in the peripheral blood. There was however no difference in the percentages of CD8 + and cells expressing HLA-DR molecules between the patients with PTB and normal healthy individuals. Table 3 shows the comparison of the absolute number of various peripheral blood lymphocyte subsets between the PTB patients and normal healthy individuals. The absolute numbers of NK cells (p = 0.000) and the B lymphocytes (p = 0.02) were significantly higher than the normal individuals. No significant difference in the absolute numbers of the rest of the lymphocyte subsets could be detected. The mean helper suppressor ratio found in the patients with PTB (1 ± 0.4) was significantly lower (p = 0.006) when compared with the normal healthy individuals (1.4 ± 0.4) data not shown.

Table 2. Comparison of percentages of peripheral blood lymphocyte subsets in patients with pulmonary tuberculosis and normal healthy individuals.

S. No	Lymphocyte Subset	Patients with PTB % (mean ± s.d)	Normal Healthy Individuals % (mean ± s.d)	P value
1.	T lymphocytes (CD3+)	55.9 ± 11.3	76.2 ± 5.7	0.0000
2.	Helper lymphocytes (CD4+)	30.8 ± 10.7	44.1 ± 5.4	0.0000*
3.	Cytotoxic lymphocytes(CD8+)	34.2 ± 9.9	33.1 ± 7.5	0.7190
4.	Natural killer (NK) cells(CD56+CD16+)	28 ± 12.8	9.9 ± 5.6	0.0000*
5.	Activated lymphocytes(HLA-DR+)	14.9 ± 8.3	13 ± 5	0.4010
6.	B lymphocytes	18.2 + 8	11 + 2.5	0.0000*

PTB = Pulmonary Tuberculosis (n = 10), Normal Individuals (n = 25). S.d = standard deviation, * = statistically significant.

Table 3. Comparison of absolute numbers of peripheral blood lymphocyte subsets in patients with pulmonary tuberculosis and normal healthy individuals.

S. No	Lymphocyte subset	Patients with PTB absolute number (mean ± s.d) /µl	Normal healthy individuals absolute number (mean ± s.d) /µl	P value
1.	T lymphocytes (CD3+)	1064.3 ± 675.5	1354.4± 244.4	0.0670
2.	Helper lymphocytes(CD4+)	542.9 ± 350.3	775.7 ± 225.4	0.0250*
3.	Cytotoxic lymphocytes(CD8+)	34.2 ± 9.9	33.1± 7.5	0.7190
4.	Natural killer (NK) cells (CD56+CD16+)	523.7 ± 360.9	177.1 ± 133.7	0.0000*
5.	Activated lymphocytes(HLA-DR+)	250.6 ± 178.4	248.8± 96.4	0.9690
6.	B lymphocytes(CD19+)	426.8 + 452	205.7 + 69	0.0250*

PTB = Pulmonary tuberculosis (n = 10), normal healthy Individuals (n = 25), s.d = standard deviation, * = statistically significant.

DISCUSSION

Alterations in the peripheral blood lymphocyte subsets were detected in this study. The most notable findings were increased percentage with an absolute numbers of NK and B cells while reduced percentage with an absolute numbers of CD4 + lymphocytes in the peripheral blood of patients with PTB. A variety of immune abnormalities of the peripheral blood lymphocyte subsets including NK cells in PTB have already been described in PTB (Snyder et al., 2007; Barcelos et al., 2008) . CD4 + T-cells have been shown to play a vital role in the control of MTB infection, while the role of other cells, such as CD8 + T-cells and γδ T cells, is still controversial (Flynn et al., 2000). It is primarily due to the conflicting reports of the lymphocyte subsets abnormalities in PTB that there has been no agreement on understanding the mechanisms underlying the disease process. This issue is further complicated by the fact that peripheral blood lymphocyte subsets undergo changes in response to treatment with anti-TB drugs (Veenstra et al., 2006).

Several studies in the recent past have focused on the role of NK cells in PTB. NK cells have been shown to lyse MTB- infected monocytes and alveolar macrophages (Vankayalapati et al., 2005). This has been attributed to the NK cells promoting the production of IFNγ by CD8 + cells (Vankayalapati et al., 2004) . Production of IFNγ is believed to be an important host event in defense against MTB (Stenger and Modlin, 1999), as decreased synthesis of IFNγ has been associated with active tuberculosis (Zhang et al., 1995). A

subset of NK cells with $CD3^-CD16^-CD56^+$ phenotype has been identified to have a capacity to produce high levels of IFNγ along with other cytokines (Cooper et al., 2001; Bantoni et al., 2005).

A higher percentage of granzyme A $CD56^+$ cells have also been reported in PTB (Vidyarani et al., 2007) signifying the relevance of the GzmA-mediated pathway of apoptosis in immunity against MTB. Collectively these data suggest that NK cells may be pivotal for handling MTB infection. Significantly higher numbers and percentage of $CD3^-CD16^+CD56^+$ NK cells detected in patients with PTB in this study may therefore indicate the contribution of NK cells in the host immune response against MTB infection.

The immune response after MTB infection and disease may be assessed by the measurement of T-lymphocyte phenotypes in the human peripheral blood. Decreased numbers of CD4 + and CD8 + T-cells in patients with active tuberculosis have been reported in several studies (Beck et al., 1985; Onwubalili et al., 1987; Singhal et al., 1989; Jones et al., 1997). In this study decreased CD4 + counts and percentage were observed in patients with PTB, whereas the CD8 + cells were no different when compared with the normal controls. The reduction in CD4 + cell counts may have resulted in a significantly decreased helper suppressor ratio observed in patients with PTB. CD4+ lymphopenia has been considered as an indicator of disease activity to an extent that depletion of CD4 + cells has been correlated with the severity of the disease process (Jones et al., 1997; Kony et al., 2000). This is further supported by the fact the CD4 counts have been shown to return to normal after successful treatment of tuberculosis (Rodrigues et al., 2002). The CD3 + lymphocyte counts though reduced, but failed to achieve statistical significance. The percentage was however significantly lower in patients with PTB in the present study. It is possible that the depletion of CD4 + cells may have mostly contributed to the decreased of CD3 + cell percentage and counts in patients with PTB. The lymphocyte cytotoxic effect of CD8 + cells is strongly related to the host capacity to block the development of disease due to MTB (Flynn et al., 1992). The normal CD8 + cell counts observed in the present study may therefore indicate a better immune status of the patients in this study.

Immunity against MTB comprises of a predominant cellular response and majority of researchers have dismissed the participation of B cells in defending against the infection. B cells and antibodies are thought to offer no significant contribution towards protection against MTB (Kumararatne, 1997). However experiments in mice have unveiled the role of B cells in the optimal host response against MTB by increasing the infection inoculum (Vordermeier et al., 1996; Maglione et al., 2007). In addition the protection of mice against MTB infection by administration of intravenous immunoglobulin (IVIG) suggests further impact of humoral immunity upon host defense in tuberculosis (Roy et al., 2005). The higher B cell counts and the percentage observed in the present study may therefore have an important bearing on the disease process. The role of B cells in PTB requires further investigation as decreased B cell counts in the peripheral blood have also been reported in patients with active PTB (Corominas et al., 2004).

Expression of HLA-DR molecule is generally regarded as an activation marker. No difference in the expression of HLA-DR molecule on CD3 + cells could be detected in the present study. However a higher percentage of HLA-DR positive CD3 + cells in the peripheral blood of patients with PTB has recently been reported (Aktas et al., 2009) . It is difficult to interpret the discrepancy as the presence of activated CD3 + in the peripheral blood may indicated systemic host response in PTB which was not detected in the present study.

Conclusion

The peripheral blood lymphocyte alterations in the present study particularly the increased levels of NK and B cells in PTB patients, emphasizes the need for further investigations to evaluate their role in pulmonary tuberculosis. Similarly, MTB associated CD4 + lymphopenia observed in the present study and in other studies require further investigation to gain a better understanding of the mechanism(s) and factors involved.

REFERENCES

Aktas E, Ciftci F, Bilgic S, Sezer O, Bozkanat E, Deniz O, Citici U, Deniz G (2009). Peripheral Immune Response in Pulmonary Tuberculosis. Scand. J. Immunol., 70: 300-308.

Bancroft G (1993). The role of natural killer cells in innate resistance to infection. Curr. Opin. Immunol., 5: 503-510.

Barcelos W, Sathler-Avelar R, Martins-Filho OA, Carvalho BN, Guimara˜es TMPD, Miranda SS (2008). Andrade HM, Oliveira MHP, Toledo VPCP. Natural Killer Cell Subpopulations in Putative Resistant Individuals and Patients with Active Mycobacterium tuberculosis Infection. Scand. J. Immunol., 68: 92-102.

Batoni G, Esin S, Favilli FPM, Bottai D, Maisetta G, Florio W, Campa M (2005). Human CD56bright and CD56dim natural killer cell subsets respond differentially to direct stimulation with Mycobacterium bovis bacillus Calmette-Gue´rin. Scand. J. Immunol., 62: 498-506.

Beck JS, Potts RC, Kardjito T, Grange JM (1985). T4 lymphopenia in patients with active pulmonary tuberculosis. Clin. Exp. Immunol., 60: 49-54.

Biron CA, Nguyen KB, Pien GC Cousens LP, Salazar-Mather TP(1999). Natural killer cells in antiviral defense: function and regulation by innate cytokines. Annu. Rev. Immunol., 17: 189-220.

Blythe MJ, Zhang Q, Vaughan K de Castro R Jr, Salimi N, Bui HH, Lewinsohn DM, Ernst JD, Peters B, Sette A (2007). An analysis of the epitope knowledge related to Mycobacteria. Immunome. Res., 3: 10.

Boom WH, Canaday DH, Fulton SA, Gehring AJ, Rojas RE, Torres M (2003). Human immunity to M. tuberculosis: T cell subsets and antigen processing. Tuberculosis, 83(1-3): 98-106.

Brill KJ, Li Q, Larkin RCDH, Kaplan DR, Boom WH, Silver RF (2001). Human natural killer cells mediate killing of intracellular Mycobacterium tuberculosis H37Rv via granule-independent mechanisms. Infect. Immun., 69: 1755-1765.

Calvelli T, Denny TN, Paxton H, Gelman R, Kagan J (1993). Guideline for flow cytometric immunophenotyping: A report from the National

Institute of Allergy and Infectious Diseases, Division of AIDS. Cytometry, 14(7): 702-15.
Campos-Martin Y, Gomez del Moral M, Gozalbo-Lopez B, Suela J, Martinez-Naves E (2004). Expression of human CD1d molecules protects target cells from NK cell-mediated cytolysis. J. Immunol., 172: 7297-7305.
Cooper MA, Fehniger TA, Turner SC, Chen KS, Ghaheri BA, Ghayur T, Carson WE, Caligiuri MA (2001). Human natural killer cells: a unique innate immunoregulatory role for the CD56bright subset. Blood, 97: 3146-3151.
Corominas M, Cardona V, Gonzalez L, Caylà JA, Rufi G, Mestre M, Buendia E (2004). B lymphocytes and co-stimulatory molecules in *Mycobacterium tuberculosis* infection. Int. J. Tuberc. Lung. Dis., 8: 98-105.
Flynn JL, Ernst JD (2000). Immune responses in tuberculosis. Cur. Opinion. Immunol., 12: 432-436.
Flynn JL, Goldstein MM, Triebold KJ, Koller B, Bloom BR (1992). Major histocompatibility complex class I-restricted T cells are required for resistance to *Mycobacterium tuberculosis* infection. Proc. Natl. Acad. Sci., 89: 12013-7.
Glassroth J (2004). Tuberculosis 2004: challenges and opportunities. Trans. Am. Clin. Climat. Assoc., 116: 293-310.
Jacobsen M, Mattow J, Repsilber D, Kaufmann SH (2008). Novel strategies to identify biomarkers in tuberculosis. Biol. Chem., 389(5): 487-495.
Jiang Y, Shang H, Zhang Z, Diao Y, Dai D, Geng W, Min ZM, Han X, Wang Y, Liu J (2005). Alterations of natural killer cell and T-lymphocyte counts in adults infected with human immunodeficiency virus through blood and plasma sold in the past in china and in whom infection has progressed slowly over a long period. Clin. Diagn. Lab. Immunol., 12: 1275-1279.
Jones BE, Oo MM, Taikwel EK, Qian D, Kumar A, Maslow ER, Barnes PF (1997). CD4 cell counts in human immunodeficiency virus-negative patients with tuberculosis. Clin. Infect. Dis., 24: 988-991.
Kony SJ, Hane AA, Larouze B, Samb A, Cissoko S, Sow PS, Sané M, Maynart M, Diouf G, Murray JF (2000). Tuberculosis-associated severe CD4+ T-lymphocytopenia in HIV -seronegative patients from Dakar SIDAK Research Group. J. Infect., 41: 167-171.
Kumararatne DS (1997). Tuberculosis and immunodeficiency--of mice and men. Clin. Exp. Immunol., 107: 11-14.
Kunst H (2006). Diagnosis of latent tuberculosis infection: the potential role of new technologies. Resp. Med., 100: 2098-2106.
Maglione PJ, Xu J, Chan J (2007). B cells moderate inflammatory progression and enhance bacterial containment upon pulmonary challenge with Mycobacterium tuberculosis. J. Immunol., 178: 7222-7234.
Onwubalili JK, Edwards AJ, Palmer L (1987). T4 lymphopenia in human tuberculosis. Tubercle., 68: 195-200.
Pilheu JA, De Salvo MC, Gonzalez J, Rey D, Elias MC, Ruppi MC (1997). CD4+ T-lymphocytopenia in severe pulmonary tuberculosis without evidence of human immunodeficiency virus infection. Int. J. Tuberc. Lung. Dis., 1: 422-426.
Raviglione MC, Narain JP, Kochi A (1992). HIV-associated tuberculosis in developing countries: clinical features, diagnosis, and treatment. Bull. WHO., 70: 515-526.
Rodrigues DSS, Medeiros EAS, Weckx LY, Bonnez W, Salomao R, Kallas EG (2002). Immunophenotypic characterization of peripheral T lymphocytes in *Mycobacterium tuberculosis* infection and disease. Clin Exp. Immunol., 128: 149-154.
Roy E, Stavropoulos E, Brennan J, Coade S, Grigorieva E, Walker B, Dagg B, Tascon RE, Lowrie DB, Colston MJ, Jolles S (2005). Therapeutic efficacy of high-dose intravenous immunoglobulin in Mycobacterium tuberculosis infection in mice. Infect. Immun., 73: 6101-6109.
Singhal M, Banavalikar JN, Sharma S, Saha K (1989). Peripheral blood T lymphocyte subpopulations in patients with tuberculosis and the effect of chemotherapy. Tubercle., 70: 171-178.
Snyder-Cappione JE, Nixon DF, Loo CP, Chapman JM, Meiklejohn DA, Melo FF, Costa PR, Sandberg JK,Rodrigues DS, Kallas EG (2007). Individuals with Pulmonary Tuberculosis Have Lower Levels of Circulating CD1d-Restricted NKT Cells. J.Infec. Dis., 195: 1361-134.
Stenger S, Modlin RL (1999). T cell mediated immunity to *Mycobacterium tuberculosis*. Curr. Opin. Microbiol., 2: 89-93.
Vankayalapati R, Garg A, Porgador A, Griffith DE, Klucar P, Safi H, Girard WM, Cosman D, Spies T, Barnes PF (2005). Role of NK cell-activating receptors and their ligands in the lysis of mononuclear phagocytes infected with an intracellular bacterium. J. Immunol., 175: 4611-4617.
Vankayalapati R, Klucar P, Wizel B, Weis SE, Samten B, Safi H, Shams H, Barnes PF (2004). NK cells regulate $CD8^+$ T cell effector function in response to an intracellular pathogen. J. Immunol., 172: 130-137.
Veenstra H, Baumann R, Carroll NM, Lukey PT, Kidd M, Beyers N, Bolliger CT, van Helden PD, Walzl G (2006). Changes in leucocyte and lymphocyte subsets during tuberculosis treatment; prominence of CD3dimCD56+ natural killer T cells in fast treatment responders. Clin. Exp. Immunol., 145: 252-260.
Vidyarani M, SelvarajP, Jawahar MS, Rajeswari ND, Anbalagan S, Narayanan PR (2007). Intracellular granzyme A expression of peripheral blood lymphocyte subsets in pulmonary tuberculosis. Cur. Sci., 93: 823-825.
Vordermeier HM, Venkataprasad N, Harris DP, Ivanyi J (1996). Increase of tuberculous infection in the organs of B cell-deficient mice. Clin. Exp. Immunol., 106: 312-316.
Zhang M, Lin Y, Lyer DV, Gong J, Abrams JS, Barners PF (1995). T cell cytokine responses in human infection with *Mycobacterium tuberculosis*. Infect. Immun., 63: 3231-3234.