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Research Article

Changes in Cytokines Status of Patients with Recurrent Pulmonary Tuberculosis Receiving Chemotherapy

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Background and objective

Select cytokines in patients with recurrent TB receiving anti-TB chemotherapy were compared to the levels in healthy individuals in order to assess the balance between inflammatory and anti-inflammatory responses.

Materials and methods

Cytokines, IL-2, IL-4, IL-8, IL-10 and IFN-γ, were examined by quantitative ELISA (pg/L) in patients with recurrent TB (1st group; N=100) and healthy individuals (2nd group; N=30). All patients received standard TB drugs: Isoniazid (0.3 g); Rifampicin (0.6 g); Pyrazinamide (2 g); Ethambutol (1.2 g) and/or Streptomycin (1 g).

Results

At baseline the 1st group had serum levels of IL-2 (39.44±0.71); IL-4 (9.55±0.24); IL-8 (21.14±0.32); IL-10 (40.04±0.74) and IFN-γ (106.20±0.67); 2nd group had IL-2 (21.60±0.80); IL-4 (29.99±1.27); IL-8 (9.96±0.62); IL-10 (50.25±1.26); IFN-γ (63.82±2.27). After 2 months, there was a significant decrease in pro-inflammatory cytokine levels in the 1st (IL-2: 29.59±0.55; IL-8: 18.22±0.22; IFN-γ: 71.14±1.21). Conversely, IL-4 increased in 1st to 16.68±0.44 and IL-10 – 48.53±0.87 ($p<0.05$).

Conclusion

Prior to the study initiation patients with TB had higher IL-2, IL-8, IFN-γ and lower IL-10 and IL-4 content than healthy controls. Two-month chemotherapy produced significant reduction in pro-inflammatory cytokines and increase in anti-inflammatory IL-10 and IL-4, with levels approaching those of healthy controls. Thus, TB drugs appear to have the anti-inflammatory effect in TB patients, which was predictive of positive clinical outcome.

Keywords: Recurrent Pulmonary Tuberculosis; Cytokines; Interleukin-2; Interleukin-4; Interleukin-8; Interleukin-10; Interferon-γ

Introduction

Tuberculosis (TB) occupies one of the leading positions among the most common infectious diseases. Despite the stabilization of morbidity, there is a difficult epidemiological situation with TB due to the increasing of proportion of severe advanced forms and rate growth of relapse in the total incidence of TB [1,2]. In addition, the issue of pulmonary

tuberculosis relapse has been remaining of great importance due to the consistently high incidence of tuberculosis reactivation in patients with active tuberculosis history for many years [3].

The body immune system, in which lungs perform a significant role, provides systemic and local reactions to antigenic impact of different genesis. Cell interactions in the immune response are provided by cytokines system. Cytokines — are secretion products of cells involved in immune processes to identify cellular interaction. They provide pro- and anti-inflammatory, immunostimulatory, immunosuppressive, hematopoietic effects, acting through receptors on the of target cells surface [4].

Studies on cytokines issues is becoming an obligatory part of immunological tests in clinical practice. The nature and intensity of the immune response is dependent on assessment of cytokine profiles, this allows obtaining information about various types of functional activity of immune cells, the severity of inflammation and its prognosis, the relationship between processes of activation of T-helper cells [5], the effectiveness of new immunological drugs [6] and the monitoring of therapy [7]. According to some authors, one of the key cytokines in tuberculosis inflammation is interleukin (IL) -2, IL-4, IL-8, IL-10 and interferon-γ (IFN-γ) [5,7,8]. It should be noted ambiguity of the mentioned above references as to cytokines markers [9-15].

The major cytokine producing lymphocytes are T-helpers (Th). IL-2 is produced by activated Th1, cytokine that stimulates an immune response by activating T-cell populations, stimulates the synthesis of interferon-γ and tumor necrosis factor (TNF) [16,17], is a factor of growth and differentiation of B-lymphocytes involved in implementation immune defense and antitumor resistance [16,17]. In anti-TB protecting it participates mainly in delayed-type hypersensitivity reactions, activates cytotoxic T cells, monocytes and macrophages that induce the synthesis and secretion of TNF-α, IL-6, IL-8 [16].

Product of Th 2 cells — IL-4 — is a strong growth factor for B-lymphocytes, which contributes to their differentiation, activation and reproduction, supports cell proliferation, promotes the development of allergic reactions has antitumor activity [16-18].At the same time IL-4 induces cytotoxic activity of macrophages, stimulating their migration the inflammation site, increases toxicity of TNFα resulting in pulmonary fibrosis development [17,19].

IL-8 — low molecular-weight inflammatory cytokine is produced under the influence of bacterial endotoxins and cytokines, mainly TNF and IL-1, contributes to monocytes and neutrophils activation, and induces their chemotaxis to the inflammation site [20,21].

IL-10 is produced by Th2 lymphocytes type, as well as by monocytes, macrophages, is the most important regulator of immune response that suppresses the activity of macrophages and Th1 cells and ensures the implementation of some biological effects of Th2. IL-10 takes part in anti-inflammatory response realization by autotolerance inhibiting [22].IL-10 promotes the development of humoral component of the immune response providing anti-parasitic protection and allergic reactivity [22].

IFNγ is formed by stimulating T-lymphocytes with various antigens [23], it is a key cytokine for cellular immune response and humoral immune response inhibitor [24].

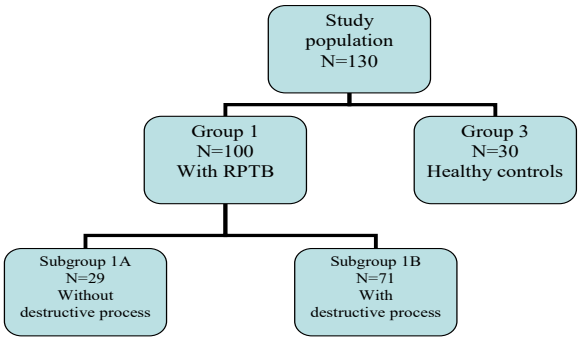
Monitoring the diversity of subpopulations of lymphocytes provides a variety of cytokine production and determines the preference of Th1 or Th2 depending on the type of cellular response to Mycobacterium tuberculosis [5,25]. T-helper cells are the first which identify antigenic peptide, after receiving activating signal they differentiate into Th1 and Th2 types [26,27]. Th1 activation leads to the formation of cellular immunity, and activation of Th2 mediates humoral immunity [28]. Th1 mainly produces IL-2 and IFNγ, and Th2 produces IL-4 and IL-10 [29,30,31,32]. Many of antibacterial agents, including anti-TB drugs, have certain immunosuppressive effects that limit the potential of the body to resist infections [33].

Thus, the aim of our study was to establish the characteristics of immunological activity of serum cytokines in patients with relapsed pulmonary tuberculosis (RPTB) before and during antimycobacterial chemotherapy.

Materials and Methods

There were 130 persons under our observation, of them 100 patients with RPTB (1st group) and 30 relatively healthy donors (2nd group). The first group was divided into two subgroups: 1A RPTB patients without a destructive process in the lung (29 patients), 1B — with the presence of destructive process (71 patients) (Figure 1).

Figure 1. Composition of study population according to underlying diagnosis.



Patients were selected for this study when staying in the Regional TB Hospital № 1 and in the Regional Anti-TB Dispensary № 1 both located in Kharkiv; in the Regional Anti-TB Dispensary № 3 in Zmiyiv and in the Regional Anti-TB Dispensary № 4 in Izyum, both located in Kharkiv region. The healthy donors (2nd group) with comparable age characteristics, with no lung disease in anamnesis, no pathological changes in the X-ray lung examination of the chest, with no chronic infectious disease, without acute allergic reactions and respiratory diseases along 3 preceding the study months. The first-line drugs were used as a basic anti-TB chemotherapy: isoniazid (0.3 g), rifampicin (0.6 g), pyrazinamide (2 g), ethambutol (1.2 g) and/or streptomycin (1 g) with further decreasing of the dose regime.

Cytokines Measurement

The levels of cytokines (IL-2, IL-4, IL-10 and IFNγ) in serum were evaluated by using standard ELISA kits (Company «VECTOR-BEST», Novosibirsk, Russian Federation). Serum samples for testing were taken during first days on admission and after 2 months of in-patient treatment.

Statistical Evaluation

The obtained data were statistically analyzed by standard Student t-test [34]. The difference was considered to be significant at $p<0.05$.

Ethics

The project was approved by the Ethics Committee of the Kharkiv National Medical University, Ukraine. It was conducted according to the Declaration of Helsinki standards. All of the patients provided written informed consent and explicitly provided permission for blood analyses, as well as for the collection of relevant clinical data.

Results

Prior to treatment in patients with RPTB, there was estimated a significant increase of IL-2, IL-8 and IFN-γ levels when compared to healthy donors (Table 1). This can be due to the universal reaction of the immune response to the Mycobacterium tuberculosis (MBT) agents destruction. It is well-known that the role of IL-2 and IFN-γ in antituberculous protection is provided by its effect on the macrophages activation, direct cytotoxicity of T-cells [16]. On the other hand, it supports pro-activation of phagocytic destruction MBT [23].

During the study of anti-inflammatory cytokines such as IL-4 and IL-10, we observed their significantly lower levels in blood serum comparing to the 2nd group at the beginning of the treatment ($p<0.05$). Thus, activity status and there are only a tendency for the profile of Th2- lymphocytes were reduced before treatment. This can be due to higher activation and the number Th1-lymphocytes, confirmed by increased antituberculous immunity in patients with pulmonary TB in the beginning of chemotherapy.

IL-2, IL-8 and IFN-γ blood concentrations significantly decreased in patients with pulmonary TB after the two-month standard therapy when compared to basic levels. IL-2, IL-8 and IFN-γ levels were still significantly higher in patients with pulmonary TB after two months of treatment when compared to the 2nd group. Judging by this reaction, we can conclude of activity and quantitative Th1-lymphocyte population decrease in this cohort of patients. In its turn, this may indicate the relative process stabilization in patients with pulmonary TB due to significantly higher levels of IL-2 and IFN-γ comparing to healthy donors.

While we observed IL-2, IL-8 and IFN-γ concentrations decrease during the conducted two-month therapy in patients with pulmonary TB, L-4 and IL-10 levels, by contrast, were significantly increased in patients with TB when compared to the baseline parameters before chemotherapy. Thus, we could observe activation and are only a tendency for the profile increasing the Th2-lymphocytes amount and as a result — relative Th1-lymphocytes stabilization development. Comparing the described parameters to those of healthy donors after two-month anti-TB treatment, IL-4 levels were significantly lower than in the 2nd group, indicating relative insufficiency of this parameter restoration at the 2nd month of treatment ($p<0.05$). As for IL-10 concentration, there were observed non-significant values in patients with RPTB, indicating relative cytokine restoration at the 2nd months of the therapy ($p>0.05$) (Table 1).

When comparing parameters in subgroups of the RPTB patients, we observed insignificancy of mentioned above results at the beginning and after two months of antimycobacterial chemotherapy that may indicate independent immune response, both in the presence or absence of the destructive process.

Thus, in patients with RPTB we observed a significant increase of IL-2, IL-8, IFN-γ concentrations and decrease of IL-4, IL-10 levels when compared to healthy donors. When using standard antimycobacterial therapy we observed significant decreased IL-2, IL-8, IFN-γ levels, and, on the contrary, under the influence of anti-tuberculosis therapy IL-4, IL-10 levels significantly increased. No significant difference in cytokine levels was revealed between the presence and absence of cavities, both before or after two months of the treatment.

Table 1. Cytokines in patients with pulmonary tuberculosis prior to and after 2-month anti-TB chemotherapy as compared with healthy controls (Mean ± SD), pg/L.

Groups and subgroups		**1st group** relapsed pulmonary tuberculosis (N=100)	**1A subgroup** without TB destructive process (N=29)	**1B subgroup** with TB destructive process (N=71)	**2nd group** controls (N = 30)
Interleukin-2	Before treatment	39.44±0.71*	37.93±1.34*#	40.06±0.83*#	21.60±0.80
	After 2 months of treatment	29.59±0.55*•	28.64±0.91*•#	29.97±0.68*•#	
Interleukin-4	Before treatment	9.55±0.24*	10.07±0.44*#	9.33±0.29*#	29.99±1.27
	After 2 months of treatment	16.68±0.44*•	17.61±0.77*•#	16.30±0.54*•#	
Interleukin-8	Before treatment	21.14±0.32*	21.38±0.53*#	21.05±0.40*#	9.96±0.62
	After 2 months of treatment	18.22±0.22*•	18.24±0.33*•#	18.21±0.27*•#	
Interleukin-10	Before treatment	40.04±0.74*	39.95±1.48*#	40.07±0.86*#	50.25±1.26
	After 2 months of treatment	48.53±0.87®•	48.10±1.36®•#	48.70±1.10®•#	
Interferon-γ	Before treatment	106.20±0.67*	107.20±1.34*#	105.70±0.77*#	63.82±2.27
	After 2 months of treatment	71.14±1.21*•	73.43±2.34*•#	70.21±1.41*•#	

® – discrepancy is not significant (p>0.05) when compared with Group 2;

*– discrepancy is significant (p<0.05) when compared with Group 2;

•– discrepancy is significant (p<0.001) when compared before treatment and after two months levels among Group and Subgroups;

#– discrepancy is not significant (p>0.05) between Subgroups 1A and 1B.

Conclusions

1. Patients with pulmonary TB display signs of inflammation characterized by decrease of IL-4 and IL-10 and increase of IL-2, IL-8 and IFN-γ as compared to healthy donors.

2. Standard 2-month TB therapy results in reversal of inflammation characterized by decrease in IL-2 and IFN-γ and increase of IL-10 to the levels comparable to healthy donors.

3. IL-2, IL-4, IL-8, IL-10, and IFN-γ are immune correlates of treatment outcome and can help to identify better strategy for TB management.

4. TB chemotherapy may have immunomodulatory effect of anti-inflammatory nature.

5. No significant difference in cytokine (IL-2, IL-4, IL-8, IL-10, and IFN-γ) levels was revealed between the presence and absence of cavities, both before or after two months of the treatment.

References

1. World Health Organization. Global tuberculosis report 2014: WHO report 2014, Geneva Switzerland, 2014.

2. World Health Organization. Guidelines for surveillance of drug resistance in tuberculosis. Geneva, Switzerland: WHO, 2009.

3. Petrenko V M, Cherenko S O, Byalyk I B. Evolution and effectiveness of antimycobacterial therapy in patients with pulmonary tuberculosis relapse. Ukrainian pulmonology journal. 2009, 3: 14-19.

4. Chernushenko E F. Current problems in immunology of tuberculosis and pulmonology. Ukrainian pulmonology Journal. 2003, 3: 94-96.

5. Kulpraneet M, Sukwit S, Sumransurp K et al. Cytokine production in NK and NKT cells from Mycobacterium tuberculosis infected patients. Southeast Asian J Trop Med. Public Health. 2007, 38 (2): 370-375.

6. Sakhno L V, Tikhonova M A, Ostanin A A et al. Interleukin-2 in the correction of T-cell anergy in patients with pulmonary tuberculosis. Probl Tuberk Bolezn Legk. 2006, (1): 48-52.

7. Chernushenko E F, Kadan L P, Panasiukova O R et al. Cytokines in the evaluation of the immunity system in patients with lung tuberculosis. Ukrainian pulmonology journal . 2010, 2: 39-43.

8. Pospelov A L, Auerbach M M, Gubkin M F. The level of synthesis of IFN-γ, TNF-α, IL-1β and IL-10 at different stages of treatment of tuberculosis in children and adolescents. Tuberculosis and Lung Disease. 2011, 8: 36-40.

9. Winkler S, Necek M, Winkler H et al. Increased specific T cell cytokine responses in patients with active pulmonary tuberculosis from Central Africa. Microbes Infect. 2005, 7(9-10): 1161-1169.

10. Veenstra H, Baumann R, Lukey P T et al. High levels of intracellular IL-4 are expressed in circulating apoptotic T cells in patients with tuberculosis and in community controls. Tuberculosis (Edinb). 2008, 88(1): 21-30.

11. Verbon A, Juffermans N, Van Deventer S J H et al. Serum concentrations of cytokines in patients with active tuberculosis (TB) and after treatment. Clin Exp Immunol. 1999, 115 (1): 110-113.

12. Roberts T, Beyers N, Aguirre A et al. Immunosuppression during active tuberculosis is characterized by decreased interferon- gamma production and CD25 expression with elevated forkhead box P3, transforming growth factor- beta , and interleukin-4 mRNA levels. J Infect Dis. 2007, 195(6): 870-878.

13. Smith S M, Klein M R, Malin A S et al. Decreased IFN-gamma and increased IL-4 production by human CD8(+) T cells in response to Mycobacterium tuberculosis in tuberculosis patients. Tuberculosis. 2002, 82 (1): 7-13.

14. Martha Torres, Teresa Herrera, Hector Villareal et al. Cytokine Profiles for Peripheral Blood Lymphocytes from Patients with Active Pulmonary Tuberculosis and Healthy Household Contacts in Response to the 30-Kilodalton Antigen of Mycobacterium tuberculosis. Infect Immun. 1998, 66 (1): 176-180.

15. Torres M, Herrera T, Villareal H et al. Cytokine profiles for peripheral blood lymphocytes from patients with active pulmonary tuberculosis and healthy household contacts in response to the 30-kilodalton antigen of Mycobacterium tuberculosis. Infect Immun 1998; 66 (1): 176-80.

16. Berezhnaya N M, Chekhun V F. Immunology of malignant growth. Kiev.2005, 791.

17. Petrenko V I, Varchenko Y A. The role of cytokines and use them to immunotherapy in patients with pulmonary tuberculosis. Tuberculosis, pulmonary diseases, HIV infection. 2010, 2(02): 78-85.

18. Rook G A. Th2 cytokines in susceptibility to tuberculosis. Curr Mol Med. 2007, 7 (3): 327-337.

19. Demyanov A V, Kotov A Yu, Simbirtsev A S. The diagnostic value of the study of cytokine levels in clinical practice. Cytokines and inflammation. 2003, 2 (3): 20-35.

20. Boggaram V, Gottipati K R, Wang X et al. Early secreted antigenic target of 6 kDa (ESAT-6) protein of Mycobacteriumtuberculosis induces interleukin-8 (IL-8) expression in lung epithelial cells via protein kinase signaling and reactive oxygen species. J Biol Chem. 2013, 288(35): 25500-11.

21. Salina T Iu, Morozova T I. The characteristics of production of cytokines in patients with tuberculosisof lungs. Klin Lab Diagn. 2013, (3): 18-20.

22. Marino J H, Wiele C J, Everhart I M et al. Attenuation of cytokine responsiveness during T cell development and differentiation. J. Interferon Cytokine Res . 2006, 26(10): 748-759.

23. Kadagidze Z G. Cytokines. Practical oncology. 2003, 4 (3): 131-139.

24. Ferand R A, Bothamley G H et al. Interferon-Gamma Responses to ESAT-6 in Tuberculosis Patients Early into and After Anti-Tuberculosis Treatment. International Journal of Tuberculosis of Lung Diseases. 2005, 9 (9): 1034-1039.

25. Tiul'kova T E, Chugaev Iu P, Kashuba E A. The specific features of immune system functioning in tuberculous infection. Probl Tuberk Bolezn Legk. 2008, (11): 48-55.

26. Ferraz J C, Melo F B, Alluquerque M F et al. Immune factors

and immunoregulation in tuberculosis. Braz J Med Biol. 2006, 11 (39): 1387-1397.

27. Stenger S. Immunological control of tuberculosis: role of tumour necrosis factor and more. Ann Rheum Dis. 2005, 64 (4): 24-28.

28. Them M L, Hope I C, Mcaulay M et al. The effect of tuberculin testing on the development of cell-mediated immune responses during Mycobacterium bovis infection. Vet Immunol. and Immunopathol 2006, 114 (1-2): 25-36.

29. Ellner J J, Waillis R S. Immunologic aspects of mycobacterial infections. Rev Infect Dis. 1989, 11(2) 455-459.

30. Shams S A, Zavaran H A, Shaghasempour S et al. IL-10 and IL-12 production in response to Mycobacterium tuberculosis total lipid antigens in multidrug resistant tuberculosis. Iran J Immunol. 2010, 7(1): 57-63.

31. Tan Q, Xie W P, Min R et al. Characterization of Th1- and Th2-type immune response in human multidrug-resistant tuberculosis. Eur J Clin Microbiol Infect Dis. 2012, 31(6): 1233-1242.

32. Rook G A. Th2 cytokines in susceptibility to tuberculosis. Curr Mol Med. 2007, 7 (3): 327-337.

33. Chukanov VI, Kaminskaia GO, Livchane E. Frequency and pattern of adverse reactions due to treatment of patients with pulmonary tuberculosis with antitubercular reserve drugs. Probl Tuberk Bolezn Legk. 2004, 10: 6-10.

34. Lapach S N, Chubenko A V, Babich P N. Statistical methods in biomedical studies using Excel, Kiev. 2000, p. 320.