Lymphocyte Subsets in Patients with İdiopathic Dilated Cardiomyopathy

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Objective: Although chronic myocardial inflammatory process mediated by viral and autoimmune factors has been postulated in the pathogenesis of idiopathic dilated cardiomyopathy (IDC), the role of autoimmune mechanisms still remains unclear. The aim of the present study was to investigate the rates of various T cell subsets and natural killer (NK) cells in peripheral blood in order to see whether they had a role in the immunoregulation of IDC.
Methods: The surface markers of peripheral T and B lymphocytes were detected and percentages of pan T and B cells as well as helper (CD4+) and suppressor (CD8+) T lymphocytes subsets in the peripheral blood and their ratio (CD4+/CD8+) were determined in 27 patients with IDC and in 20 healthy controls. NK cell percentage was also studied.
Results: There were no significant differences between IDC and control groups with respect to T and B cell percentages. The percentages of CD4+ T cell subsets were similar in both groups (48.7±8.7 % vs. 43.5±9.7 % respectively; p=0.107). CD8+ T cell percentage was significantly decreased in patients with IDC than in controls (22.6±7.7 % vs. 28.2±8.2 %, respectively; p=0.044). CD4+/CD8+ ratio was markedly higher in patients with IDC than controls (2.6±1.8 vs. 1.6±0.6, respectively; p=0.006). There was no significant difference in the NK cell percentage between groups.
Conclusion: Decreased CD8+ T cell subset is the cause of increased CD4+/CD8+ ratio, which may imply decreased self-tolerance and an immunoregulatory defect in the pathogenesis of IDC. *(Ana Kar Der, 2001; 1: 98-100)*
Key words: Idiopathic dilated cardiomyopathy, T lymphocyte subsets

Introduction

Idiopathic dilated cardiomyopathy (IDC) is a chronic heart disease characterized with progressive dilatation and loss of function of ventricles in the absence of known causes (1). The most common manifestation of disease is heart failure due to impaired diastolic and systolic ventricular function (1, 2). Although chronic myocardial inflammatory process mediated by viral and autoimmune factors have been considered in the etiopathogenesis of IDC, the role of autoimmune mechanisms still remains unclear (3-7). T lymphocytes are classified as 'helper' T cells, which express CD4+, cell surface marker and as 'suppressor' T cells that express CD8+ cell surface marker. Natural killer (NK) cells are potent cytotoxic cells that are not antigen specific and play a role in antiviral defense mechanism (6). Some previous studies showed significant imbalance between helper and suppressor T lymphocyte subsets and decrease in NK cell activity in peripheral blood samples of patients with IDC, but the results of these studies are conflicting (8-12). Kanda et al. (8) reported high percentage of CD4+ and low CD8+ T cells in IDC patients when compared to healthy controls and patients with ischemic heart disease, but the results of Klappacher et al. (10) did not confirm these findings. Besides, it is not clear whether those immunological features are causes or consequences of the myocardial inflammation (1).

The aim of the present study was to determine the changes in the percentage of T cell subsets and NK cells in peripheral blood in order to investigate the role of cellular immunoregulation in patients with IDC.

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Material and Methods

Twenty-seven patients with IDC (10 male, 17 female; mean age: 44.9±12.7 years) and 20 healthy controls (10 male, 10 female; mean age 47.2±6.1 years) were included in the study. Patients and control subjects were matched by age and gender. Five patients with IDC were in functional class II, 16 were in functional class III and 6 were in functional class IV according to the criteria of New York Heart Association.

The diagnosis of dilated cardiomyopathy was done according to the criteria that were recommended by World Health Organization and the National Heart, Lung and Blood Institute (13, 14). All patients had left ventricular dilatation (end-diastolic diameter index >2.7 cm/m2) and impaired systolic contraction (left ventricular ejection fraction <40% or fractional shortening <25%). The patients who had coronary artery disease, active myocarditis, systemic arterial hypertension, specific primary or secondary heart muscle disease, isolated right ventricular dilatation, and valve or pericardial disease were excluded from the study.

Two-dimensional and Doppler echocardiographic studies using Toshiba SSH 1.60 A system with 3.75 MHz pulse wave transducer were done in all patients. Tracings of end diastolic and end systolic left ventricular contours were obtained using two-chamber apical view approach for calculation of left ventricular volumes (Simpson method) and ejection fraction.

The Epic-profile I Coulter flow cytometry (Epics Division of Coulter Corporation, Miami, Florida, USA) was used to determine the percentages of the pan-T (CD2+), T-helper subset (CD4+), and T-suppressor subset (CD8+) of the T-lymphocyte and pan-B (CD20+) lymphocytes as well as the NK cells (CD56+) from the peripheral blood of patients with IDC and healthy controls. CD4+/CD8+ ratio was calculated. For this purpose, 1 cc of venous blood was taken from each patient and control and poured into a tube containing ethylene diamine tetra acetic salt (EDTA). For each type of cell, 100 microliters (mL) of blood were placed into a 12x75 mm test tube and 10 mL of a suitable monoclonal antibody solution was added. The following monoclonal antibodies were used in the study: T11-RD1/B1-FITC (Coulter code-2524R133), T4-RD11/T8FITC (Coulter code-2224E243) and NKH-1-RD1 (Coulter code-2933J103).

The samples were incubated with antisera for 10 minutes at room temperature. After the samples were placed in the Coulter Multi-Q-Prep Instrument, 600 mL of immunoprep A (Erythrocyte Lytic Agent), 265 mL of immunoprep B (leukocyte stabilizer) and 100 mL of immunoprep C (cell membrane fixative) were added. The preparations were transferred one by one to the flow cytometry instrument and the percentages of all of the parameters were determined.

SPSS package (SPSS Inc., 1998, Chicago, Illinois) for Windows (version 9.00) was used for statistical analysis. Statistical analysis was performed by Mann-Whitney U test. Results are presented as mean±SD. A value of $p<0.05$ was accepted as significant.

Results

The means and statistical comparisons among groups are represented in Table 1. There are no significant differences between IDC and control groups with respect to pan-T and B cell percentages. The percentages of CD4+ T cell subsets are not different in both groups (48.7±8.7 % vs. 43.5±9.7%, respectively; p=0.107). The mean CD8+ T cell percentage of IDC group is significantly lower than that of control group (22.6±7.7 % vs. 28.2±8.2 %, respectively; p=0.044). CD4+/CD8+ ratio is markedly higher in patients with IDC than control subjects (2.6±1.8 vs. 1.6±0.6, respectively; p=0.006). There is no significant difference in NK cell percentage between groups.

Tablo-1: Percentages (mean±SD) of lymphocyte subsets in patients with idiopathic dilated cardiomyopathy (IDC) and in controls.

	IDC (n=27)	Controls (n=20)	p values
Pan T (%)	79.4±10.3 M=84.0	84.2±4.2 M=84.9	0.307
Pan B (%)	12.9±6.0 M=11.4	10.8±3.4 M=10.7	0.458
CD4+ (%)	48.7±8.7 M=51.2	43.5±9.7 M=42.1	0.107
CD8+ (%)	22.6±7.7 M=23.2	28.2±8.2 M=28.5	0.044
CD4+/CD8+	2.6±1.8 M=2.1	1.6±0.6 M=1.5	0.006
NK (%))	12.7±10.5 M=9.7	14.3±6.0 M=14.8	0.277

M: Median

Disccussion

Previous clinical and experimental data suggest that autoimmune mechanisms can play a role in the pathogenesis of IDC (9). Various immunological abnormalities such as decreased activity and percentage of CD4+ and CD8+ T cells as well as NK cells has been reported in the pathogenesis of IDC (8,11,12,15,16).

We found significantly elevated CD4+/CD8+ cell ratio as a result of decreased CD8+ levels in patients with IDC when compared to those in controls in our study. These results are in accordance with some other studies (8, 9). On the other hand, the results of Klappacher et al. (10) did not confirm these findings. Most of the previous studies have shown increased CD4+ and decreased CD8+ T cell subsets in peripheral blood of patients with IDC and an increase in CD4+/CD8+ ratio (8, 9, 11). The imbalance in helper and suppressor T cells and increased CD4+ cell percentages may result in increased helper or decreased suppressor T cell activity. This inappropriate cell function may lead to excessive inflammatory response to culprit antigens by decreasing self-tolerance and may play a role in the development of IDC (9). Alterations in T cell subsets and NK cells can mediate various reactions such as the delayed hypersensitivity-type (DHT) reaction. That reaction can be initiated by CD4+ T lymphocyte recognition of foreign antigen presented by antigen-presenting cells (17). Influences of CD4+ T cells and DHT reaction can lead to resultant secretion of cytokines such as interleukin (IL)-2 and IL-10 (6, 18). IL-2 causes proliferation of antigen-activated T cells and has autocrine effects stimulating the synthesis of cytokines by T cells, include IL-2 itself and tumor necrosis factor-alpha. These mediators may participate in reversible and irreversible tissue injury (6).

CD4+/CD8+ ratio is increased in our patients with IDC. Our data support the previous studies that have found an immunoregulatory defect in IDC; but the results of these studies are not sufficient to conclude whether this immunoregulatory defect is the cause or the consequence of IDC. Abnormalities in cellular and humoral immunity have been recognized in both myocarditis and IDC. However causative relation of these findings has not been demonstrated (3-7).

In conclusion, decreased CD8+ T cell subset is the cause of increased CD4+/CD8+ ratio, which may imply decreased self-tolerance and an immunoregulatory defect in the pathogenesis of IDC.

References

1. Dec GW, Fuster V. Idiopathic dilated cardiomyopathy. N Engl J Med 1994; 331: 1564-75.
2. Wynne J, Braunwald E. The cardiomyopathies and myocarditis. In Braunwald E, ed. Heart Disease. 5th ed. Philadelphia: WB Saunders Company; 1997. p.1404-63
3. Magnusson Y, Wallukat G, Waagstein F, Hjalmarson A, Hoebeke J. Autoimmunity in idiopathic dilated cardiomyopathy. Circulation 1994; 89: 2760-7.
4. Marriott JB, Goldman JH, Keeling PJ, Baig MK, Dalgleish AG, McKenna WJ. Abnormal cytokine profiles in patients with idiopathic dilated cardiomyopathy and their asymptomatic relatives. Heart 1996; 75: 287-90.
5. Kühl U, Noutsias M, Seeberg B, Schultheiss HP. Immunohistological evidence for a chronic intramyocardial inflammatory process in dilated cardiomyopathy. Heart 1996; 75: 295-300.
6. Barry WH. Mechanisms of immune-mediated myocyte injury. Circulation 1994; 89: 2421-32.
7. Caforio ALP, Keeling PJ, Zachara E, Mestroni L, Camerini F, Mann JM. Evidence from family studies for autoimmunity in dilated cardiomyopthy. Lancet 1994; 344: 773-7.
8. Kanda T, Yokoyama T, Ohshima S, et al. T-lymphocyte subsets as noninvasive markers of cardiomyopathy. Clin Cardiol 1990; 13: 617-22.
9. Smalcelj A, Durakovic Z, Batinic D, Bogdan I, Grgic V. Relation between helper and suppressive/cytotoxic T lymphocytes in the peripheral blood in patients with dilated cardiomyopathy. Acta Med Yugosl 1991; 45: 3-13 (abst.)
10. Klappacher G, Mehrabi M, Franzen P, Plesch K, Binder M, Haab D. Endomyocardial HLA expression is increased to the same extent in idiopathic and secondary dilated cardiomyopathy. Immunol Lett 1994; 41: 59-66.
11. Gerli R, Rambotti P, Spinozzi F, et al. Immunologic studies of peripheral blood from patients with idiopathic dilated cardiomyopathy. Am Heart J 1986; 112: 350-5.
12. Anderson JL, Carlquist JF, Hammond EH. Deficient natural killer cell activity in patients with idiopathic dilated cardiomyopathy. Lancet 1982; 2: 1124-7.
13. Brandenberg RO, Chazov E, Cherian G. Report of the WHO/ISFC task force on definition and classification of the cardiomyopathies. Circulation 1981; 64: 37A-8A.
14. Manolio TA, Baughman KL, Rodeheffer R. Prevalence and etiology of idiopathic dilated cardiomyopathy. Am J Cardiol 1992; 69: 1458-66.
15. Yokoyama A. Natural killer cells in dilated cardiomyopathy. Tohoku J Exp Med 1988; 154: 335-44 (abst).
16. Kanda T, Ohshima S, Yuasa K, Watanabe T, Suzuki T, Murata K. Idiopathic myocarditis associated with T-cell subset changes and depressed natural killer activity. Jpn Heart J 1990; 31: 741-4.
17. Abbas AK, Lichtman AH, Pober JS. Cellular and Molecular Immunology. Philadelphia, Pa: WB Saunders Co, 1991.
18. Hasdai D, Scheinowitz M, Leibovitz E, Sclarovsky S, Eldar M, Barak V. Increased serum concentrations of Interleukin-1 beta in patients with coronary artery disease. Heart 1996; 76: 24-8.