Immunological Evidence In Idiopathic Dilated Cardiomyopathy

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ABSTRACT:

BACKGROUND:

Immunological factors in the pathogenesis of idiopathic dilated cardiomyopathy (IDC) were suggested previously on the basis of the demonstration of mononuclear cell infiltrates and autoantibodies against the myocardium.

OBJECTIVE:

Was to determine the changes in the percentage of T cell subsets in peripheral blood in order to investigate the role of cellular immunoregulation in patients with idiopathic dilated cardiomyopathy.

PATIENT AND METHOD:

The surface markers of peripheral T and B lymphocytes were detected and percentages of helper (CD4+) and suppressor (CD8+) T lymphocytes subsets in the peripheral blood and their ratio. (CD4+/CD8+) were determined in 62 patients with IDC and in 50 healthy controls.

RESULTS:

There were no significant differences between IDC and control groups with respect to CD4+ T cell subsets (p=0.4). CD8+ T cell percentage was significantly decreased in patients with IDC than in controls (p=0.005). CD4+/CD8+ ratio was markedly higher in patients with IDC than controls (p=0.005).

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.

KEY WORDS: idiopathic dilated cardiomyopathy, t lymphocyte subsets

INTRODUCTION:

Idiopathic dilated cardiomyopathy (IDC) is a collective term for diseases of unknown cause in which signs and symptoms are due primarily to systolic dysfunction and cardiac dilatation leading to low-output states. The condition was defined by the World Health Organization as an often severe dilatation of one or both ventricles with impaired systolic function and invariably accompanied by hypertrophy. A number of factors have been implicated in the causation of Idiopathic dilated cardiomyopathy (IDC), including alcohol,[1] nutritional deficiency,[2] postpartal state [3] , and viral infections.[4] The toxic drugs potentially pluricausal nature Idiopathic dilated cardiomyopathy (IDC) often requires a diagnosis of exclusion lead to difficulties in studying IDC. IDC patients were shown to carry both immunoglobulins bound to myocardial tissue[5,6] and serum autoantibodies with and without organ specificity.[7,8,9] on the other hand, only a few studies with conflicting results have dealt with alterations in cell-mediated immune mechanisms. Whereas some investigators observed a reduction of $CD8^+/CD11^-$ cytotoxic and an increase of $CD4^+/2H4^+$ suppressor/inducer peripheral-blood T cells in patients with IDC,[10] others have demonstrated a defect in suppressor cell function in vitro.[11,12] In another report, significantly increased numbers of both T-cell subpopulations were shown to be $HLA\text{-}DR^+$ and thus activated.[13] Sachs et al[14] reported an elevated percentage of T suppressor cells, whereas Anderson et al[15] observed no different frequencies of T-cell subpopulations. Also, it has been proposed that viral myocarditis can progress to IDC as a consequence of an autoimmune reaction.[16,17,18] The effect of viral component via cardiac sarcolemma serving an antigenic source that direct immune response to attaché the

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myocardium. This appear to be associated with human leukocyte antigen HLA particularly DR4 suggest abnormal immunoregulation. This assumption is supported by the identification of coxsackie virus B–specific RNA sequences and infiltrating lymphocytes among damaged myocytes in heart muscle biopsy specimens from some IDC patients.[17,18,19,20].

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

PATIENT AND METHODS:

Sixty-two patients with IDC (30 male, 32 female; mean age: 44.2±10.7 years) and 50 healthy controls (20 male, 30 female; mean age 49.7±12.0 years) over the period Jan. 2001-Mar. 2011 were included in the study. Patients and control subjects were matched by age and gender.

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), the diagnosis of idiopathic Idiopathic dilated cardiomyopathy was depending on physical examination (s3, s4), atrioventriculer regurgitation, chest x-ray showed marked cardiac enlargement especially left ventricle enlargement, electrocardiography showed sinus tachycardia, atrial, ventricular arrhythmia, T wave changes and interventricular conduction defect. Echo showed 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

Phenotypic Analysis of CD_4^+ and CD_8^+ Cells by Direct Immunofluorescent Test.

1 - Principle of the test:

The direct IF technique in which the monoclonal antibodies to human CD antigen recognize specific cell surface Ag on the lymphocyte ,was used to determine the percentage of T-helper (CD4+), and T-cytotoxic (CD8+) of the T-lymphocytes from the peripheral blood of patients with IDC and healthy control.

Cell counting in this study, was done as following:

Cells /ml =the average count / square × dilution factor × 10^4.

Kept a separate count of viable and non-viable cells

Cell viability (%) = total viable cells / total cells × 100.

2 - Direct IFT: (Kit, Antihuman CD8/ RPE, Darko Denmark, No. FR883

The slides pre-coated with lymphocytes were removed from freezer, allowed to reach room temperature, unwrapped and wash with PBS. Slides were lay flat, section side up, in humidity chamber then 10µl of fluorochrom conjugated monoclonal antibodies at 1/10 dilution were added to each well in Teflon coated slide, cover chamber and slide were left undisturbed in incubator at 37°C. Slides then transferred to staining jar filled with PBS at room temperature and PBS replaced twice at 5 minutes intervals. A drop of mounting media (nine parts of glycerol to one part of 0.2 M carbonate buffer, PH=9) placed on each well of slides, to enhance fluorescence and retard fading on exposure to UV- light. Then cover slips were lowered into place slowly to avoid bubbles, cover slips may be sealed around edges with clear nail polish. Slides were examined then with fluorescence microscope in dark room. Positive CD_4^+ cell give green apple when stained with fluorescein isothocyanate (FITC) labeled antibodies and positive CD_8^+ cell give orange–red when stained with phycoerthyrin (PE) – labeled antibodies.

Statistical analysis

The χ^2 test was used to determine the variation in genotype distribution between the different groups. Statistical significance was considered as $P<0{\cdot}05$. All statistical tests were performed using SPSS statistical software package version 11 .

RESULTS:

In the current study, the frequency of T-helper and T cytotoxic subsets in reference to the total T-cell population were monitored using specifically labeled monoclonal antibodies using a direct immunofluorescent technique.

The analysis revealed no difference in mean $CD4^+$ cells compared to control group, but the frequency of $CD8^+$ cells in such patients decreased. Therefore, ratio of $CD4^+$ / $CD8^+$ significantly increased compared to control group ($p<0.05$) as shown in table-1.

Table 1: The difference in mean ratio $CD4^+$ / $CD8^+$ between the study groups

	Study groups	
Mean ±SD	Healthy control	IDC
*CD4+	1.24±0.7	1.60±0.5
**CD8+	8.5±1.5	2.7±0.5
**CD4+/CD8+	1.7±0.4	2.8±0.6

P-value:
*non significance=0.4
**Significance=0.005

DISCUSSION:

The information available on the state of the immune system in normal hearts and in hearts with IDC is conflicting. Considerable attention has been focused on the concept of immune-mediated damage as a possible causal factor in IDC, especially after acute myocarditis. An enteroviral genome can be demonstrated in 29% of patients with end-stage IDC.[47] None of the investigations of cellular events in IDC have compared peripheral-blood cells with infiltrating cells of the same patients, and the number of T-cell markers included in these studies is very limited. It has been an open issue so far whether the T cells observed in hearts with IDC are representative of a specifically disease-related, ongoing, local immune response or, alternatively, are inactive residues of an earlier immune response not directly related to IDC.[48,49,50]

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).

CONCLUSION:

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

To summarize, our findings (1) support the hypothesis that cell-mediated immunity has a role in myocyte degeneration, or destruction, in IDC and (2) argue in favor of an ongoing, chronic immune process in its pathogenesis. This is confirmed by the fact that the distribution of peripheral-blood T-cell subsets was not significantly different from that of tissue T cells, suggesting that tissue T cells from normal heart are identical to those involved in initiating and maintaining this process.

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