Study on Lymphocyte Subsets and NK cell Function in Patients with Dilated Cardiomyopathy

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An acquired defect or damage of a subpopulation of suppressor T lymphocytes is reported in connection with autoimmune diseases. In the present study, the role of immunity was examined in 7 patients with dilated cardiomyopathy (DCM). The frequency of lymphocyte subsets using monoclonal antibodies and natural killer (NK) cell activity was evaluated to determine whether DCM patients had lymphocyte abnormalities that would support the hypothesis that the pathological mechanism of DCM is an immune disturbance.

The peripheral lymphocyte counts were significantly lower in patients with DCM and higher in patients with ischemic heart disease (IHD) than in normal controls (NC) ($p < 0.01$). The percentage of T cells, B cells, OKT4 and OKT8 positive cells was not statistically different among the three groups studied here, whereas the percentage of T γ cells was significantly reduced in DCM patients ($p < 0.05$). NK cell functional activity as tested in DCM and IHD patients was frequently deficient ($22.1 \pm 19.3\%$ in DCM, $13.8 \pm 3.0\%$ in IHD, $37.4 \pm 12.7\%$ in NC).

Our results suggest that an imbalance in cellular immune reactions partly explain the pathogenesis of DCM.

THE role of immunity has been discussed in a variety of cardiac disorders. Recent reports suggest that the immune system may be involved in the pathogenesis of dilated cardiomyopathy (DCM)¹⁻³. There is some experimental evidence to support this concept^{4,5}. Yamakawa et al⁶ found a high incidence of heterophile antibodies in patients with DCM; Fukuta et al⁷ demonstrated the antibody-mediated cytotoxicity or cytolysis against cultured heart cells. Fowels et al⁸ and Eckstein

et al⁹ showed a defect in suppressor cell function in patients with DCM in vitro. Thus, some aberrant humoral and cellular response, or defects in immune regulations, may be involved in the pathogenesis of cardiomyopathy.

Recently, Anderson et al¹⁰ showed deficient natural killer (NK) cell activity in DCM patients. Increasing evidences show that NK cells are important in the immune surveillance such as natural resistance against tumors, viruses and other microorganisms, and allogenic rejection in bone-marrow transplants. Augmented NK activity might be directed to cytotoxicity against virus-infected or altered myocardial cells. Alternatively, defective activity of NK cells might be responsible for the insidious propagation of the progression of myocardial lesions after initial

Key Words:

Anti-heart antibodies
Lymphocyte subsets
Suppressor cells
Natural killer (NK) activity
Dilated cardiomyopathy

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Grant-in-Aid for Scientific Research (no. 59570363)

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Fig.1. Peripheral lymphocyte counts.

* significantly different from NC ($p < 0.01$). NC = Normal controls, DCM = Dilated cardiomyopathy, IHD = Ischemic heart disease. Shaded area shows standard deviation of the count in NC.

injury by viruses or other etiological agents.

The purpose of the present study is to evaluate the immunological conditions in patients with DCM by frequency of lymphocyte subsets and NK cell activity.

MATERIALS AND METHODS

Subjects were seven patients with DCM, ranging in age from 52 to 77 with a mean age of 64.0. Six patients with ischemic heart diseases (IHD; 4 with postmyocardial infarction and 2 with angina pectoris), ranging in age from 46 to 74 with a mean age of 60.6, were used as the disease control group. The normal control group (NC) included 15 healthy adults (age range 25-37, mean 28.1). Diagnosis was made using echocardiography, phonocardiography, cardiac catheterization, coronary angiography, right ventricular endomyocardial biopsy and other clinical methods as necessary.

Lymphocyte subsets assay: Peripheral blood lymphocytes (PBL) were isolated from heparinized venous blood on Ficoll-Hypaque density gradient and washed three times in RPMI 1640 medium. Lymphocytes positive for membrane immunoglobulins were detected by membrane immunofluorescence methods using polyclonal antihuman Ig fluorescein isothiocyanate labelled rabbit serum. T cells and T γ cells were identified using the double rosette formation method. Ox red blood cells (Eox) were fixed by 2.5%

TABLE I PERCENTAGE AND NUMBERS OF T AND B CELLS

	T cell		B cell	
	%	count/mm ³	%	count/mm ³
DCM	83.4 $\pm$ 3.5	1190 $\pm$ 359	8.0 $\pm$ 5.8	105 $\pm$ 75.6
IHD	85.6 $\pm$ 4.1	2164 $\pm$ 380	6.4 $\pm$ 1.9	162 $\pm$ 61.3
NC	85.5 $\pm$ 2.1	1670 $\pm$ 112	7.7 $\pm$ 3.2	148 $\pm$ 62.0

TABLE II PERCENTAGE AND NUMBERS OF T4⁺ AND T8⁺ CELLS

	T4 ⁺ cell		T8 ⁺ cell		T4 ⁺ /T8 ⁺ ratio
	%	count/mm ³	%	count/mm ³	
DCM	45.9 $\pm$ 10.2	673 $\pm$ 336	30.7 $\pm$ 10.9	431 $\pm$ 172	1.70 $\pm$ 0.8
IHD	52.4 $\pm$ 9.8	1336 $\pm$ 371	26.2 $\pm$ 6.3	671 $\pm$ 240	2.19 $\pm$ 0.98
NC	43.1 $\pm$ 7.7	842 $\pm$ 159	30.7 $\pm$ 9.2	607 $\pm$ 213	1.51 $\pm$ 0.56

T4⁺ = OKT4 positive cells; T8⁺ = OKT8 positive cells

TABLE III PERCENTAGE AND NUMBERS OF T γ CELL

	T γ cell	
	%	count/mm ³
DCM	9.2 $\pm$ 3.7	133 $\pm$ 70.8
IHD	15.8 $\pm$ 11.3	430 $\pm$ 352
NC	15.8 $\pm$ 7.7	312 $\pm$ 158

*significantly different from NC and IHD ($p < 0.05$).
 NC = Normal controls; DCM = Dilated cardiomyopathy; IHD = Ischemic heart disease

TABLE IV PERCENTAGE AND NUMBERS OF LEU11a⁺ LYMPHOCYTE AND NK CELL ACTIVITY

	Leu 11a ⁺		NK activity
	%	count/mm ³	% CI
DCM	10.1 $\pm$ 7.8	136 $\pm$ 96	22.1 $\pm$ 19.3
IHD	7.8 $\pm$ 8.1	182 $\pm$ 187	13.8 $\pm$ 3.0
NC	12.1 $\pm$ 10.7	239 $\pm$ 213	37.4 $\pm$ 12.7

Leu11a⁺ = Leu11a positive cell

%CI = %⁵¹Cr release (%cytotoxic index)

glutaraldehyde. Eox were washed and treated by anti-Eox rabbit IgG (EoxA-IgG) for 30 min at 37°C. EoxA-IgG treated (EoxA-IgG⁺) cells were then stained in 5% trypan blue solution and the final concentration was adjusted to 1% in RPMI 1640 supplemented with 20% fetal calf serum (FCS). EoxA-IgG⁺ cells and neuraminidase treated 1% sheep blood cells (En⁺) were mixed with 1.0×10^6 /ml PBL. The mixture was incubated at 37°C for 15 min, centrifuged at 200g for 5 min and incubated at 4°C for at least 1 hour. The pellet was gently resuspended and 200 lymphocytes were counted for determination of rosette cell. Lymphocytes with three or more erythrocytes were counted as a rosette-positive cell. The T γ subpopulation was obtained by rosetting En⁺ cells with EoxA-IgG⁺ cells.

T cell subsets and NK cells were investigated using monoclonal antibodies OKT4, OKT8(Ortho Diagnostic System), Leu7, Leu11a (Becton-Dickinson): 40 μ l of 1×10^7 mononuclear cells were incubated at 4°C for 25 min with 5 μ l of monoclonal antibodies followed by fluoresceinated goat anti-mouse Ig. The stained cells were then analyzed by using a flow cytometer (FACS III: Becton Dickinson, Mountain View,

Fig.2. Natural killer cell activity in normal controls (NC), dilated cardiomyopathy (DCM), and ischemic heart disease (IHD) patients. Shaded area shows standard deviation of the activity in NC.
 %CI = %⁵¹Cr release (%cytotoxic index)

CA) and the percent of cells showing specific staining was determined.

NK cell functional assay: K-562 cells were cultured in RPMI 1640 supplemented with 10% FCS and used as targets. They were labelled with ⁵¹Chromium (⁵¹Cr) (100 μ Ci/ 2×10^6 cells) by incubation for 1 hour at 37°C. Labelled K-562 cells were washed three times and incubated with lymphocytes in 96-well, round bottom microplates (Falcon) at effector/target ratio (E/T) of 40 : 1. After 4 hours' incubation at 37°C, 100 μ l of aliquot were removed from each well, and radioactivity was estimated in a gamma counter. The NK activity was estimated by the following formula: %⁵¹Cr release = cpm (experimental) – cpm (spontaneous release)/cpm (maximal) – cpm (spontaneous release). The values are presented as the mean of triplicate samples.

RESULTS

Peripheral lymphocyte subsets: Fig. 1 displays the peripheral lymphocyte counts that were significantly lower in patients with DCM and higher in patients with IHD than NC ($p < 0.01$). The mean values of total T cells and B cells are listed

in Table I (mean $\pm$ SD), and T cell subsets in Table II (mean $\pm$ SD). The percentage of T cells (E-rosette forming cells), B cells (surface immunoglobulin bearing lymphocytes), OKT4 and OKT8 positive cells was not statistically different among the three groups. However, the percentage of T γ cells in patients with DCM was 9.2 ± 3.7 , which was significantly reduced in comparison with IHD patients and NC (Table III, $p < 0.05$).

Number and activity of NK cell: Table IV shows the percentage of Leu11a positive cells and NK cell activity. Relative correlation between Leu11a positive cell frequency and NK cell activity was found. Fig. 2 showed distributions of NK cell activity in DCM, IHD patients and NC. NK cell activity in NC was $37.4 \pm 12.7\%$ (mean $\pm$ SD). However, most of patients with DCM and IHD had low activity ($< 20\%$) of NK cell.

DISCUSSION

Serum anti-heart antibodies (AHAb), reacting with myocardial tissue, have been found in patients with DCM, but the actual role of AHAb is not essentially clear in terms of the pathogenesis of this disease. Some authors reported a high incidence of AHAb, bound to cell membrane in the myocardium in the patients with DCM and reported their cytotoxicity to cultured heart cells^{2,6,7}. Other authors¹¹ have suggested that the autoantibodies detected by immunofluorescence probably reflect damage of cardiac muscle regardless of the cause.

It is well known, however, that the immune response is a balance of several immunological mechanisms. The presence of AHAb may be due to altered cooperation between T and B lymphocytes which results in activation of B cells or T helper or depression of T suppressor cells. Fowels et al⁸ and Eckstein et al⁹ postulated that patients with DCM had a defect in Con A induced suppressor activity. But little information is available on cellular immune function in patients with DCM. Recently, Anderson et al¹² showed suppressor deficiency in some patients with DCM as determined by immunofluorescence using monoclonal antibodies. The present study showed that in patients with DCM, peripheral lymphocyte counts were significantly reduced and the frequency of OKT4 positive cells increased and the frequency of OKT8 positive cells were not reduced. Therefore, the absolute number of OKT8 positive cells (suppressor cell compartment) might be reduced. Our results and

others^{8,9,12} showed that in patients with DCM, suppressor cells might be defective in number, or there might be reduced numbers with defective function of the cell. Further investigations are required to clarify the depletion of a subset of "cardiac antigen-specific" suppressor T cells in patients with DCM.

T γ cells are defined and characterized by the presence of a membrane receptor specific for the Fc part of IgG. We found that there was an apparent decrease of T γ cells in DCM patients. Though both T γ and OKT8⁺ subsets include cells with suppressor activity, there was a discrepancy in numbers of T γ and OKT8⁺ cells. This lack of correlation in number was not inconsistent since the two populations have been shown to be clearly different. OKT8 marker defines a T cell population which mainly includes OKT3 positive cells with suppressor or cytotoxic activities. In contrast, the precise nature of T γ cells is controversial. Reinhert et al¹³ showed that T γ cells may be non-T in origin. Recent reports¹⁴ suggest the existence of different T γ subsets belonging to the T population and having OKM1 plus OKT4 or OKT8 markers: these cells should be related to NK cells. NK and T γ cell deficiency in our results may partly support this concept.

This study was undertaken to determine whether lymphocyte abnormalities were found in patients with DCM using the hypothesis that a deficient immune mechanism is the pathogenesis of DCM. As described above, there are several indicators of a probably genetically determined immunological defects which can play a pathogenic role in DCM. But it is now impossible to say what role immune control may play in the genesis of DCM. It is speculated that reduced suppressor cell and/or increased helper cell activity, which may be induced by specifically acting pathological agents such as viruses, results in disturbed control of the B cell antibody synthesis. Such an abnormal immunological reaction may lead to insidious myocardial damage and subsequently to the development of DCM.

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