Study of Immunological Mechanism in Dilated Cardiomyopathy

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To determine the immune disturbances involved in the pathogenesis of dilated cardiomyopathy (DCM), the conditions in humoral and cellular immunity of DCM patients were studied. To examine the status in humoral immunity in DCM patients, circulating anti-heart antibodies (AHAbs) were analyzed. Circulating-AHAbs were classified into 4 types “heterophile-like”, “intra-cellular”, “intercalated disc” and “nuclear” patterns by indirect immunofluorescence technique, and a high rate of heterophile-like antibody was found in DCM. The cytotoxicity of this antibody was examined in cultured myocardial cells using the two-step method of the complement dependent cytotoxic test. The mean cytotoxic index (CI) value of the heterophile-like antibody positive sera was 22.3, which showed an apparent cytotoxicity against the cultured cells and it may be complement dependent. In addition, the frequency of lymphocyte subsets using monoclonal antibodies (OKT4, OKT8, Leu7, Leu11) and natural killer (NK) activity were evaluated to determine whether DCM patients had an imbalance in cellular immune reactions, which support the hypothesis of an immune disturbance as the pathological mechanism of DCM. The peripheral lymphocyte counts were significantly lower in patients with DCM (1737 ± 874/mm³) than in normal controls (NC, 2088 ± 556/mm³) and in patients with ischemic heart diseases (IHD, 2395 ± 469/mm³, both p < 0.01). The percentage of T, B, OKT4 and OKT8 positive cells was not statistically different among DCM, IHD, and NC groups, whereas the percentage of Tγ cells was significantly reduced in DCM patients (6.5 ± 5.0%, p < 0.05). NK functional activity as tested in DCM patients was frequently deficient (24.1 ± 16.7% in DCM, 36.7 ± 12.2% in NC). After 4-hr incubation with recombinant interleukin2 (rIL2), rIL2 induced the enhancement of NK activity in 3 out of 4 DCM patients with NK activity, although, there was a non-responder to rIL2 in this group. These results suggested that DCM patients have a low IL2 production and/or less numbers of mature cells with NK cell function.

Therefore, an imbalance in humoral and cellular immune reactions may cause insidious myocardial damage and subsequently lead to development of DCM.

Key words:
Anti-heart antibodies
Heterophile antibodies
Cytotoxicity
Lymphocyte subsets
Monoclonal antibodies
Natural killer (NK) activity
Dilated cardiomyopathy

THE role of immunity has been discussed in a variety of cardiac disorders. A preponderance of reports suggest that the immune system may be involved in the pathogenesis of dilated cardiomyopathy (DCM)\(^{1,2}\). Circulating anti-heart antibodies (AHAbs) reacting with myocardial tissue have been found in the sera of...
patients with DCM, although, the actual roles of AHAbs have not been established in terms of the pathogenesis of DCM.

It is well known that the immune response results in keeping a balance of several immunological mechanisms. The presence of AHAbs may be due to the altered cooperation between T and B lymphocytes which results in the activation of B cells, helper T cells or the depression of suppressor T cells. Thus, some aberrant humoral and cellular response or defects in immune regulations may be involved in the pathogenesis of DCM.

Recently, Anderson et al. showed deficient activity of natural killer (NK) cells in DCM patients. This defective activity of NK cell might be responsible for the insidious propagation of the progression of myocardial lesions after initial infection by viruses or other etiological agents.

The purpose of the present study is to examine the conditions of humoral and cellular immunity in DCM patients and to determine the immune disturbances involved in the pathogenesis of DCM.

MATERIALS AND METHODS

1. Humoral Immunity

We analyzed circulating AHAbs to evaluate the humoral immunity in patients with DCM.

1) Detection of circulating AHAbs

Patients: Sera from a total of 132 patients with heart diseases and from 65 normal healthy controls (NC) were collected. The patient group comprised 31 with DCM (mean age of 49.3 years) and 22 with hypertrophic cardiomyopathy (HCM, mean age of 46.8 years). The remaining 79 patients were as follows; 38 patients with myocardial infarction (MI, mean age of 63.7 years), 15 patients with angina pectoris (AP, mean age of 51.0 years) and 26 patients with rheumatic valvular disease (RVD, mean age of 55.2 years). In 65 normal subjects, the mean age was 42.6 years, which was not significantly different from that of patient groups. The diagnoses were made using echocardiography, phonocardiography, cardiac catheterization, coronary angiography, right ventricular endomyocardial biopsy and other clinical methods.

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TABLE 1  FREQUENCY OF CIRCULATING ANTI-HEART ANTIBODIES AND STAINING PATTERNS IN PATIENTS WITH HEART DISEASES

<table>
<thead>
<tr>
<th></th>
<th>Positive</th>
<th>HE</th>
<th>IC</th>
<th>Nuc.</th>
<th>ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>DCM</td>
<td>31</td>
<td>25</td>
<td>80.6*</td>
<td>20</td>
<td>64.5**</td>
</tr>
<tr>
<td>HCM</td>
<td>22</td>
<td>17</td>
<td>77.3*</td>
<td>12</td>
<td>54.5*</td>
</tr>
<tr>
<td>MI</td>
<td>38</td>
<td>22</td>
<td>57.9</td>
<td>15</td>
<td>39.5</td>
</tr>
<tr>
<td>AP</td>
<td>15</td>
<td>6</td>
<td>40.0</td>
<td>4</td>
<td>26.7</td>
</tr>
<tr>
<td>RVD</td>
<td>26</td>
<td>17</td>
<td>65.4*</td>
<td>6</td>
<td>23.1</td>
</tr>
<tr>
<td>NC</td>
<td>65</td>
<td>25</td>
<td>38.5</td>
<td>17</td>
<td>26.2</td>
</tr>
</tbody>
</table>

HE = Heterophile type; IC = Intracellular type; ID = Intercalated disc type; Nuc = Nuclear type;
DCM = Dilated cardiomyopathy; HCM = Hypertrophic cardiomyopathy.
* = significantly different from NC (p < 0.05)
** = significantly different from NC and other patient groups (p < 0.05)

Immunofluorescence procedure: Circulating AHABs were detected by an indirect immunofluorescence (IF) technique using cold ethanol fixed rat heart. The details of IF method have been described previously elsewhere.

2) Characterization of heterophile-like antibodies

Absorption of the heterophile-like antibodies positive sera: Heterophile-like antibodies positive sera were absorbed using the kit of Davidson's Absorption Test (Fuji Rebio Co.). This antibodies positive sera were mixed over 30 min with heat-treated sheep blood cells (SRBC), which contained Forssman, Paul-Bunnell and Hanganutzui-Deicher (H-D) antigens, papain-treated SRBC contained Forssman antigen, and heart-treated ox blood cells contained Paul-Bunnell and H-D antigens.

Organ distribution of the heterophile-like antigen: Heterophile staining patterns were also examined in some organs of various species using indirect IF.

3) Cell preparation and the assay of cardiocytosis:

Myocytes derived from newborn rats were cultured according to the method of Harary and Farley. The complement dependent cytotoxicity test with trypan blue was performed according to the two-step method of Amos et al. The details of these methods assessing cardiocytosis have been described elsewhere. Cytotoxicity was expressed as the cytotoxic index (CI) calculated as followed; CI = (% living cells in control system) - (% living cells in the patient serum system) / (% living cells in control system). Eleven heterophile-like antibody positive sera, 5 intracellular staining positive sera, 4 nuclear staining positive sera, 2 intercalated disc staining positive sera and circulating AHABs negative sera were used in the cytotoxic assay.

2. Cellular Immunity

Patients: Twelve patients with DCM, a mean age of 59.3 years, and 16 patients with ischemic heart disease (IHD; 13 with post MI and 3 with AP) were studied. Twenty healthy adults, a mean age of 51.3 years, were also studied as normal controls (NC).

Lymphocyte subsets assay: Peripheral lymphocytes (PBL) were isolated from heparinized venous blood on Ficoll-Hypaque density gradient and washed three times in RPMI 1640 medium. Lymphocyte positive for membrane immunoglobulins using polyclonal antihuman Ig fluorescent isothiocyanate labelled rabbit serum. T cell and Ty cell were identified by the double rosette formation method. The methods have been described previously. Ox red blood cells (Eox) fixed by glutaraldehyde were washed and treated with anti-Eox rabbit IgG (EoxA-IgG) for 30 min at 37°C. After staining in trypan blue solution, EoxA-IgG treated (EoxA-IgG+) cells were mixed with neuraminidase treated 1% sheep blood cells (En+) and PBL. After incubation at 4°C for at least 1 hour, lymphocytes with 3 or more erythrocytes were counted as rosette positive cells. The Ty subpopulation was obtained by rosetting En+ cells with Eox-IgG treated cells.

T cell subsets and NK cell were investigated using monoclonal antibodies OKT4, OKT8 (Ortho Diagnostic System), Leu7, Leu11 (Becton-
Fig. 2. a: Heterophile-like antibody staining pattern in rat heart (× 400)
b: Heterophile-like antibody staining pattern in rat stomach. The parietal cells are stained. (× 400)
c: Heterophile-like antibody staining pattern in rat kidney. The endothelium of the glomeruli and the brush borders of the proximal tubular cells are stained. (× 400)
d: Heterophile-like antibody staining pattern in rat skeletal muscle. The endomyocardium of the skeletal muscle are stained. (× 400)

Dickinson): 40 µl of 1 × 10⁷ mononuclear cells were incubated at 4°C for 25 min with 5 µl of monoclonal antibodies, followed by incubation with fluoresceinated goat anti-mouse Ig. The stained cells were then analyzed by using a flow cytometer (FACS III, Beckton Dickinson, Mountain View, CA) and the percent fraction of cells showing specific staining was determined.

NK cell functional assay: The methods have been described in previous reports in detail. NK cell function was examined by a ⁵¹Cr-specific release assay using K562 target cells. After incubation with ⁵¹Cr labelled K562 cells and PBL for 4 hours, the radioactivities from K562 cell were counted with a gamma counter. The NK activity was expressed as calculated percentage lysis by the following formula: %lysis = (E-S) / (T-S) × 100

where E is count per minute (cpm) released into experimental wells, S the spontaneous release and T the maximum cpm released by detergent (1% TritonX-100) lysis. S was always below 20% of T. The values are presented as the mean of triplicate samples.

3. Statistical Analysis
Incidence of serum cAHAbs in three groups was compared using the chi-square test. The results obtained in cellular studies were analyzed by the two-tailed Student's t-test for unpaired observations.

RESULTS

1. Humoral Immunity
The staining patterns of circulating AHAbs were classified into 4 types; "heterophile-like", "intracellular", "intercalated disc" and "nuclear" patterns (Fig. 1). In the heterophile staining pattern, the endothelium of the blood vessels and sarcolemma were stained. The intracellular pattern was characterized by intense broad striational staining and/or longitudinal fluorescence between the myofibrils (intermyofibrillar staining). The intercalated disc pattern and the nuclear pattern are also shown in Fig. 1. The predominant type of immunoglobulin associated with this antibody was IgM.
TABLE II ORGAN DISTRIBUTIONS OF THE HETEROPHILE-LIKE ANTIGENS IN VARIOUS SPECIES

<table>
<thead>
<tr>
<th>Organ</th>
<th>Species</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Rat</td>
</tr>
<tr>
<td>Heart endothelium of blood vessel</td>
<td>+</td>
</tr>
<tr>
<td>sarcolemmal region</td>
<td>+</td>
</tr>
<tr>
<td>Kidney endothelium of blood vessel</td>
<td>+</td>
</tr>
<tr>
<td>glomerulus</td>
<td>+</td>
</tr>
<tr>
<td>tubules : proximal</td>
<td>+</td>
</tr>
<tr>
<td>(brush border)</td>
<td></td>
</tr>
<tr>
<td>distal</td>
<td>+ or -</td>
</tr>
<tr>
<td>Liver endothelium of blood vessel</td>
<td>+</td>
</tr>
<tr>
<td>hepatic cell</td>
<td>-</td>
</tr>
<tr>
<td>Kupffer cell</td>
<td>+</td>
</tr>
<tr>
<td>sinusoid</td>
<td>+</td>
</tr>
<tr>
<td>Stomach endothelium of blood vessel</td>
<td>+</td>
</tr>
<tr>
<td>parietal cell</td>
<td>+</td>
</tr>
<tr>
<td>smooth muscle</td>
<td>+</td>
</tr>
<tr>
<td>Skeletal m. sarcolemmal region</td>
<td>+</td>
</tr>
<tr>
<td>Pancreas acinar cell</td>
<td>+</td>
</tr>
</tbody>
</table>

The incidence of each type of staining patterns are summarized in Table I. The occurrence of heterophile-like antibodies was 64.5% in DCM and 54.5% in HCM. These values were significantly different from those of the other patients groups and NC (p < 0.05).

The heterophile-like antibodies also produced characteristic patterns on other organ tissues. In the stomach, the parietal cells, capillaries and smooth muscle interstitium were stained (Fig. 2-b). In the kidney, the endothelium of glomeruli, the brush borders of the proximal tubular cells, and parts of the distal tubules were stained (Fig. 2-c). In the skeletal muscle, the staining pattern was similar or identical to that of heart (Fig. 2-d).

The organ distributions of the heterophile-like antigens in various species are summarized in Table II. In the liver, Kupffer cells and sinusoidal endothelium were stained by the heterophile-like antibodies positive sera. In the pancreas, acinar cells were also stained. In the heart, heterophile staining patterns were completely absorbed with heat-treated SRBC, papaine-treated SRBC and heat-treated ox blood cell.

Examination of the cytolytic activity of the heterophile-like antibodies and complement
Current Status of Dilated Cardiomyopathy

against cultured rat myocytes:

Micro-photogram of an indirect IF of the cultured myocytes treated with heterophile-like antibody positive serum is shown in Fig. 3. Fluorescence was found in the myoelminal membrane of the myocytes, while the fibroblasts were not stained.

Figure 4 shows the results of the complement dependent cytotoxic test against cultured rat myocytes. In the assay system without complement, the CI values using heterophile-like antibody positive sera were from -2.4 to 2.4%. In the experimental system with complement, the CI value using the heterophile-like antibody positive sera were 22.3 ± 7.6% (mean ± SD), whereas those using the other circulating AHAbs positive sera and negative sera were significantly lower than these using heterophile-like antibody positive sera, and scored as the negative cytotoxic activity.

The fraction of gamma globulin were obtained from the heterophile-like antibody positive sera by the method of sulphate ammonium precipitation. As shown in Fig. 5 (closed circle), the more γ-globulin concentration increased, the more the CI values augumented. After absorbing by the cultured myocytes, high levels of cytotoxic activity of the serum decreased, showing no cytotoxicity to the cells (Fig. 6). These results indicate that the cytotoxicity of the heterophile-like antibodies is specific to cultured myocytes.

2. Cellular Immunity

Peripheral lymphocyte subsets: The peripheral lymphocyte counts were significantly lower in patients with DCM (1737 ± 874/mm³) than NC (2088 ± 556/mm³, p < 0.01, Table III). The percentage of T cells (E rosette forming cells), B cells (surface immunoglobulin bearing lymphocytes), OKT4 and OKT8 positive cells were not statistically different among three groups (Table III). However, the percentage of Tγ cells in

<table>
<thead>
<tr>
<th>TABLE III NUMBERS OF PERIPHERAL LYMPHOCYTES AND PERCENTAGE OF LYMPHOCYTE SUBSETS IN THE THREE GROUPS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No.</strong></td>
</tr>
<tr>
<td>--------</td>
</tr>
<tr>
<td>DCM</td>
</tr>
<tr>
<td>IHD</td>
</tr>
<tr>
<td>NC</td>
</tr>
</tbody>
</table>

T4⁺ cell: OKT4⁺ positive cell; T8⁺ cell: OKT8⁺ positive cell
*: significantly different from IHD and NC groups (p < 0.01)
**: significantly different from NC (p < 0.05)

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TABLE IV  PERCENTAGE OF LEU 7 POSITIVE CELLS, LEU 11 POSITIVE CELLS AND NK CELL ACTIVITY IN THE THREE GROUPS

<table>
<thead>
<tr>
<th></th>
<th>Leu 7* (%)</th>
<th>Leu 11* (%)</th>
<th>NK activity (% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCM</td>
<td>16.4 ± 11.3</td>
<td>11.0 ± 9.2</td>
<td>24.1 ± 16.7</td>
</tr>
<tr>
<td>IHD</td>
<td>19.5 ± 8.4</td>
<td>11.0 ± 6.7</td>
<td>26.0 ± 12.1</td>
</tr>
<tr>
<td>NC</td>
<td>21.5 ± 9.1</td>
<td>13.3 ± 3.5</td>
<td>36.7 ± 12.2</td>
</tr>
</tbody>
</table>

*Leu 7* = Leu 7 positive; *Leu 11* = Leu 11 positive
% CI = % cytotoxic index (% 51 Cr release)

Fig.7. The effect of rIL2 on NK activity.
The cytotoxicity test after addition of rIL2 into assay system was done for 4 hrs.

patients with DCM (6.5 ± 5.0%) was significantly reduced in comparison with IHD patients (10.4 ± 6.1%) and NC (12.5 ± 6.8%, both p < 0.05).

Number and activity of NK cells: Table IV shows the percentage of Leu11 positive cells and NK cell activity. In patient groups with low frequency of Leu11 positive cells, the function of NK cell tended to show the low activity. In approximately 50% of DCM patients, NK activity was under the normal range, (24.1 ± 16.7% in DCM, 36.7 ± 12.2% in NC). The percentage of Leu7+ cells was not statistically different among three groups (Table III).

Effect of recombinant interleukin 2 on NK cell activity: Figure 7 shows the effect of recombinant interleukin 2 (rIL2) on NK cell function in 4 DCM patients with low NK activity. Mytogen free rIL2 was offered from Takeda Chemical Industries. After coculturing with PBL and various concentrations of rIL2 for 4 hrs, rIL2 induced the enhancement of NK activity in 3 of 4 DCM patients with low NK activity. The grade of NK activity boosting by rIL2 appeared to be dependent on the development of basal NK activity. However, there was a non-responder to rIL2. After stimulation by rIL2 for 4 hours, there was no increase of anti-IL2 positive lymphocytes and interferon (IFN)-γ production was noticed in all 4 specimens.

DISCUSSION

1. Status of the humoral immune reponse—Circulating anti-heart antibodies
The staining patterns of circulating AHAbs were examined by an indirect IF test using rat heart fixed in cold ethanol. The staining patterns were classified into 4 types, "heterophile-like",...
“intracellular”, “intercalated disc” and “nuclear”. The intracellular patterns contained striational and/or intermyofibrillar pattern.

Recently, Nicholson et al. have proposed a new classification for staining patterns of circulating AHABs. They differentiated between heart/muscle specific AHAb and non-tissue specific antibodies. The former may give “peripheral”, “diffuse” “striational” and “intercalated disc” patterns.

Our heterophile-like antibodies stained the endothelium of blood vessels and sarcolemmal regions. These antibodies were completely absorbed with heart-treated SRBC, papain-treated SRBC and heart-treated ox blood cell. The antibodies also stained the rat brush borders of the proximal tubular cells and smooth muscle of the stomach, Kupfer cell and sinusoidal endothelium of the liver, and acinar cells of the pancreas. These antibodies were, thus, not tissue specific. These results indicated that our antibodies might be of the H-D heterophilic type. The antibodies seemed to be identical with those described by some authors. Cossio et al. also have reported an antibody reacting with the sarcolemmal of myofibers and the plasma membranes of endothelial cells in Chagas’ diseases using an indirect IF technique, and named this antibody the endothelial-vascular-interstitial (EVI) antoantibodies. The EVI factor may be identical to our heterophile-like antibodies because of the staining pattern by as revealed indirect IF methods.

In our study, DCM patients had a high prevalence of heterophile-like antibodies. Das et al. have reported that cardiomypathic heart tissue showed abundant sarcolemmal and subsarcolemmal staining under direct IF technique. We have also found the deposition of immunoglobulin (Ig) on the sarcolemmal region using the sections of myocardium obtained by endomyocardial biopsy from DCM patients. However, it was impossible to clarify whether the deposition of Ig was specific or not, because the diffusion of the plasma component produced a non-specific IF staining.

This study demonstrated that the heterophile-like antibody induced cytolysis in the presence of a complement. The cytotoxic effect of the antibody and complement against cardiac cells may be due to a defective control of the cell membrane osmolarity, that may be influenced by an interference in the Na-K ATPase activity as reported by Friedman and Laufer. Cossio et al. have speculated that the EVI antibody may interfere with transmembrane diffusion and transport process, that is, this antibody may have a pathogenic effect. Sterin-Borda et al. have also shown that the EVI antibody induced morphologic and functional alteration in rat myocardial cells in vitro, while Maish and his coworkers demonstrated that the anti-myoelmmal antibody had cytotoxic activity in the presence of a complement against adult rat cardiocytes. These data suggest that heterophile-like antibodies may play a role in the pathogenesis in DCM. However, further investigation is required to clarify the difference between these antibodies. At present, the antigenic stimuli responsible for the formation of heterophile-like antibodies in DCM still remain to be determined. If the heterophile-like antibody plays a role in the pathogenesis of myocardial damage, a heterophile-like antigen must exist on the myocardial cell surface. We could not find this antigen in the normal adult human heart. We propose that the antigen may be released from infectious agents as in Chagas’ disease or from the patient's own tissues during the pathologic process. Recently, heterophile antigen and antibody have been demonstrated in the pathologic sera and tissue by Nishimaki et al. They have reported that this heterophile antigen might be produced as a novel antigen by pathologic tissue. This antigen was composed of several antigenic molecules and some of them might exist as normal tissue components and possibly increase during a pathologic process. Furthermore, this antigen may be released from infectious agents containing the same antigenic molecules. Recently, Löwer et al. reported that the majority of normal human sera contained naturally occurring heterophile antibodies that reacted with the carbohydrate moieties of retrovirus envelope antigens, and that such antibodies might possess both virus neutralizing and cytotoxic activity, and regulate the humoral immune response directed against infectious virus envelope glycoproteins.

2. Status in the cellular immune response

Circulating AHABs, reacting with myocardial tissue, have been found in patients with DCM. However the actual role of AHABs is not essentially clear in terms of the pathogenesis of this disease. Some authors report a high incidence of AHABs, bound to cell membrane in the myocardium in the patients with DCM and their cytotoxicity to cultured heart cells. Other

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authors\(^{19}\) have suggested that the autoantibodies detected by IF probably reflect the damage of cardiac muscle regardless of the cause.

It is well known that the immune response results in keeping a balance of several immunological mechanisms. The presence of AHAbs may be due to altered cooperation between helper T or depression of suppressor T cells. Fowels et al\(^{20}\) and Eckstein et al\(^{21}\) postulated that patients with DCM had a defect in Con A induced suppressor activity. Recently, Anderson et al\(^{22}\) showed suppressor deficiency in some patients with DCM as determined by immunofluorescence using monoclonal antibodies. Our present study showed that in DCM patients, peripheral lymphocyte counts were significantly reduced, while the frequency of OKT4+ and OKT8+ cells was not statistically different among DCM, IHD and NC groups. Therefore, absolute number of OKT8+ cells (suppressor/cytotoxic cell compartment) might be reduced in patients with DCM. Our results and others\(^{20\text{-}22}\) 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. Specific immune response to myocardial tissues should be required to recognize a cardiac antigens. These results could not always mean the defective “antigen-specific” suppressor cells. Further investigation is required to clarify the depletion of a subset of “cardiac antigen-specific” suppressor T cells in patients with DCM.

\(\Gamma\) cells are defined and characterized by the presence of a membrane receptor specific for the Fc part of IgG. We found that there was the apparent decrease of \(\Gamma\) cells in DCM patients. Though both \(\Gamma\) and OKT8+ subsets include cells with suppressor activity, there was discrepancy in numbers of \(\Gamma\) 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+ cells with suppressor or cytotoxic activities. In contrast, the precise nature of \(\Gamma\) cells is controversial. Recent reports\(^{23}\) suggest the existence of different \(\Gamma\) subsets belonging to the T population and having OKM1 plus OKT4 or OKT8 markers; these cells should be related to NK cells. NK and \(\Gamma\) cell deficiency in our results may support partly this concepts.

We found also that NK activity were significantly lower in patients with DCM than NC. NK activity has been reported to be reduced in other autoimmune disease, and NK function appears important in a natural defence against viral infection, immunosurveillance against malignancy and control mechanism of immune responses through their production of lymphokines. Anderson et al\(^3\) presented three possibilities with relation to abnormal NK activity in certain patients with DCM. First, both cardiomyopathy and NK deficiency may be due to a common pathologic process but are not related to each other in causal fashion. Secondly, cardiomyopathy may be the cause of NK deficiency. And finally, NK deficiency may be a causal factor in cardiomyopathy. Distinguishing NK deficiency as cause or effect in DCM will require further studies.

The rIL2 induced the enhancement of NK activity, although there was a non-responder to rIL2. The results of the present study demonstrate importance of IL2 for the cytolytic activity of human NK cells, and this lymphokine was able to activate NK cells in a very rapid way. IL2 is a highly potent factor for human NK cell activity and may act in cooperation with other factors like IFN to achieve the total activity in vivo. After 4-hour-stimulation with rIL2, the IL2 receptor (IL2R) on PBL recognized by the anti-IL2R antibody unchanged, and there was no IFN production by stimulated PBL (data not shown). These results suggest that DCM patients had a low IL2 production and/or less number of mature cells with NK cell function.

Recently Lanier et al\(^{24}\) have characterized the expression of Leu7 and Leu11 antigens on blood lymphocyte subsets, Leu7+ 11−, Leu7+ 11+ and Leu7− 11+ cells with different levels of NK functional capabilities and showed that the Leu7− 11+ cells had the most potent NK function. It may be thought that these subpopulations represent sequential stages of human NK cell differentiation. In our DCM patients with low NK activity, there might have been a disturbance of maturation of NK cells.

The mechanism by which IL2 augments NK activity is not well understood. It has been suggested that the enhancement of NK activity induced by IL2 is mediated by the IFN-\(\gamma\) produced in the culture. However, it has been recently reported that the augmentation of NK activity by rIL2 is not entirely dependent on IFN-\(\gamma\) production. Seki et al\(^{25}\) suggested that rIL2 might enhance the kinetics of lysis of Leu11− cells after weak binding stage. Wright et al\(^{26}\) reported that IFN could augment the production
of NK cytotoxic factors, and that these factors binding to the target cell membrane and mediated target cell lysis. However, it remains uncertain whether rIL2 has similar effects in the production of soluble factors and enhances cytoxicity.

As described above, there are several indicators of a probably genetically determined immunological defects which can play a pathologic role in DCM. However, it is now impossible to say what role immune control may play in the genesis of DCM. It is speculated, as shown in Fig. 8, that reduced suppressor cell and/or increased helper cell activity, which may be induced by specifically acting pathological agents such as viruses and/or factors which may be determined genetically, results in disturbed control of B cell antibody synthesis. The excess B cell activations may cause abnormal production of pathogenic antibodies. In addition, NK deficiency may reduce resistance to viral infection in host and this result in failing to remove virus infected cells in acute stage. In consequence, exposure of viral associated antigen or altered self antigen on the surface of myocytes may be persistant and so the specific immune mechanism to these antigens lead to further insidious myocardial damage.

Our results suggest that an imbalance in humoral and cellular immune reactions may cause insidious myocardial damage and subsequently lead to development of DCM.

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