Immunological Disorders in Patients with Dilated Cardiomyopathy

With Special Reference to the Production of Interleukin-2 and the Expression of Interleukin-2 Receptors in the Patients’ Peripheral Blood Lymphocytes

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SUMMARY

Cell-mediated immunological function was studied in 18 patients with dilated cardiomyopathy (DCM) making use of peripheral blood lymphocytes (PBL). The results were as follows:

1) Blastoid transformation of PBL stimulated with phytohemagglutinin (PHA) was significantly suppressed in patients with DCM compared with normal controls (p<0.01).

2) The T-cell subset ratio of Leu-3a-positive cells to Leu-2a-positive cells was significantly higher in patients with DCM than that in the control group (p<0.05).

3) Interleukin-2 (IL-2) production of PBL under PHA stimulation was enhanced significantly in patients with DCM (p<0.05).

4) IL-2 receptor-positive cells were significantly fewer in number in patients with DCM than in controls (p<0.05).

These results suggest that immunological disorders including changes in the IL-2 system are present in some patients with DCM and may play a role in the occurrence of DCM.

Additional Indexing Words:

Dilated cardiomyopathy  Immunological disorder  Suppressor T-cell  Helper T-cell  Interleukin-2  Interleukin-2-receptor

Dilated cardiomyopathy (DCM) is a disease of the heart consisting of a deficit in contractility accompanied by dilatation of the left ventricle, the cause of which is unknown.

It has recently been pointed out that DCM is associated with impaired function of cell-mediated immunity, such as reduced function of suppressor T-cells,11–31 reduced activity of natural killer (NK) cells41 and reduced ex-
pression of IL-2 receptors (IL-2R), and the relationship between such impaired cell-mediated immunity and the pathogenesis of DCM has currently been receiving a great deal of attention. IL-2 is a lymphokine which selectively activates T-cells, and plays an important role in the complicated immune-controlling system. IL-2 production has been studied in various autoimmune diseases, but not in DCM.

In this paper, we report on an in vitro study of changes in IL-2 production of PBL in patients with DCM.

**Subjects and Methods**

Eighteen patients with DCM were studied, 15 males and 3 females ranging in age from 25 to 61 years (average: 47±9 years). All fulfilled the diagnostic criteria of DCM described by the cooperative study group for idiopathic cardiomyopathy sponsored by the Japanese Ministry of Welfare. The 18 cases were evaluated according to the New York Heart Association classification of cardiac function; 4 cases were in grade I, 8 cases in grade II, 4 cases in grade III and 2 cases in grade IV.

Age and sex matched healthy subjects served as controls in the 4 studies.

1) Blastoid transformation of lymphocytes stimulated with PHA

Subjects included 11 patients and 11 healthy controls. Peripheral blood lymphocytes (PBL) were separated from heparin-added venous blood by specific-gravity centrifugation using Ficoll-Hypaque. After washing twice with Hanks solution (Nissui Pharm. Co. Ltd.), these PBL were suspended in RPMI 1640 (Nissui Pharm. Co. Ltd.) containing 10% heat-inactivated fetal calf serum (FCS, Gibco) to give 1×10^6 cells/ml. One hundred µl of RPMI 1640 containing 10% FCS were then added to 100 µl of this cell suspension in a U plate (Nunc.), each in triplicate. After addition of 1 µg/ml of PHA, 200 µl of the cell suspension were incubated at 37°C in a humidified 5% CO₂ atmosphere for 48 hours, then pulsed with 0.05 µci/1 µl of ³H-thymidine (³H-TdR) and incubated for an additional 24 hours. After the cells were harvested, ³H-TdR uptake was determined with a liquid scintillation spectrometer (Packard). The results were expressed as averaged cpm and the ratio of this count to those for samples incubated without PHA (stimulation index [SI]) was calculated.

2) The ratio of Leu-3a-positive cells to Leu-2a-positive cells (Leu-3a/Leu-2a)

Fourteen patients and 14 healthy controls were studied. Ten µl of a reagent containing double monoclonal antibodies of FITC-labeled anti-Leu-2a, and phycoerythrin-labeled anti-Leu-3a (Becton, Dickinson Overseas Inc.)
were added to 100 μl of PBL separated as described previously, and 200 cells each stained in green (Leu-3a-, Leu-2a+) and in red (Leu-3a+, Leu-2a-) were counted under a fluorescent microscope (Zeiss). The ratio of Leu-3a to Leu-2a was then calculated.

3) IL-2 production

Lymphocytes from 11 patients with DCM and 11 normal controls were collected and incubated for 72 hours using the same method as that given in 1). A 100 μl aliquot of the supernatant of this incubated lymphocyte suspension including 1×10^5 cells was obtained and the IL-2 activity was determined by the method of Gills et al. Briefly, the IL-2-dependent T-cell line (CTLL-2) was washed 3 times with Hanks solution and the number of cells was adjusted to 5×10^4/ml in the culture medium of RPMI 1640 with FCS. One hundred μl of the supernatant of the above incubated suspension were added to this 100 μl of CTLL-2 suspension and the total sample volume of 200 μl prepared in this way was subjected to assay. This preparation was incubated at 37°C in a 5% CO2 atmosphere for 20 hours, exposed to 3H-TdR pulses and incubated for a further 4 hours. The cells were then collected by a cell harvester and 3H-TdR uptake was determined with a liquid scintillation spectrometer and expressed as averaged cpm. At the same time, the proliferation of CTLL-2 by the addition of serial concentrations of recombinant IL-2 (Shionogi Pharm. Co. Ltd.) from 10^{-2} IU/ml to 10^{4} IU/ml was determined to give a standard curve for IL-2 activity. The IL-2 production in the test sample was read on the standard curve.

### Table I. Blastoid Transformation in Patients with DCM and Normal Controls

<table>
<thead>
<tr>
<th>Patient</th>
<th>Control</th>
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<tbody>
<tr>
<td>PHA (−)</td>
<td>PHA</td>
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<tr>
<td>1</td>
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<tr>
<td>10</td>
<td>473</td>
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Mean±SD: 774±197 cpm | 25610±20299 cpm | 34.8±28.8 | 996±617 cpm | 65293±54870 cpm | 75.3±25.0

P values (vs. controls) <0.02, <0.01.
4) Determination of IL-2 receptor (IL-2R)-positive cells

PBL obtained from 8 patients with DCM and 7 normal controls were incubated with PHA for 48 hours. The activated PBL were washed with Hanks solution, and then allowed to react with FITC-labeled anti-IL-2R antibody (Becton, Dickinson Overseas Inc.) at 4°C for 45 min. After being washed twice with Hanks solution, the PBL were suspended in 1 ml of Hanks solution. The fluorescence intensity on the surface of the cells was determined using flow-cytometry (Epics V cell sorter) and the percentage of fluorescence-positive cells was calculated.

5) Statistical analysis

The results are expressed as mean±SD and were analyzed by the Student’s t-test, p<0.05 being taken to indicate a significant difference.
RESULTS

1) Blastoid transformation of lymphocytes with PHA

Blastoid transformation of PBL in DCM patients and normal controls was examined. The $^3$H-thymidine uptake of PBL of DCM patients incubated for 72 hours with PHA was 25,610 cpm and that of PBL incubated without PHA was 774 cpm, showing a SI of 34.8±28.8. The corresponding values in healthy controls were 65,293 cpm and 996 cpm, respectively, with a SI of 75.3±25.0 (Table I). Blastoid transformation of PBL by PHA was significantly suppressed in cases of DCM (p<0.01).

2) Leu-3a/Leu-2a ratio

The absolute numbers of Leu-3a and Leu-2a-positive cells were not measured, only the ratio was investigated. As shown in Fig. 1, the Leu-3a/Leu-2a ratio for cases of DCM varied widely and abnormally high values
were seen in 5 of the 14 cases. The mean ratio for cases of DCM was 2.74 ± 1.02 and that for normal cases was 2.08 ± 0.49, giving a statistically significant difference (p < 0.05).

3) IL-2 production

Next, IL-2 production of PBL stimulated by PHA was examined in DCM patients and compared with normal controls. The degree of IL-2 production of PBL in cases of DCM varied and the mean value was 7.0 ± 3.5 U/ml, while that in normal controls was 3.9 ± 2.8 U/ml. The value was significantly higher in patients with DCM (p < 0.05) (Fig. 2).
4) IL-2 receptor (IL-2R)

As shown in Fig. 3, the rates of IL-2R-positive cells in PBL stimulated with PHA were $27.4 \pm 13.4\%$ for cases of DCM and $48.7 \pm 12.8\%$ for normal controls, with a statistically significant difference ($p<0.05$). In 2 cases of DCM with low percentages of IL-2R-positive cells (11.7% and 20.9%), high IL-2 production (11 U/ml and 8.4 U/ml) as well as low proliferative response (SI 40.3, SI 8.1) was relatively inadequate. No relationship between clinical severity of the disease and the immunological abnormality was found in any of our experiments.

**Discussion**

DCM is defined as a disease associated with reduced myocardial contractility caused by unknown factors. Since several putative causes have been advocated including myocarditis, alcohol abuse, pathological changes in myocardial small vessels and immunological disorders, DCM is considered to be heterogenous in its pathogenesis. In the present study we found that in DCM, cell-mediated immunity was impaired as reflected in the decreased expression of IL-2R and augmented production of IL-2.

Impaired cell-mediated immunity in DCM was first reported by Das et al in 1976. They described that the blastoid transformation of lymphocytes treated with PHA was markedly suppressed in 6 of 14 cases of DCM. The results of our study were in line with those of Das et al.

Recent introduction of monoclonal antibodies has made it possible to investigate lymphocyte surface markers using laser-flow cytometry. For DCM it has been reported by many investigators that OKT8-positive cells, suppressor/killer T-cells, were decreased in PBL, resulting in an increment of the ratio of OKT4-positive cells, helper/inducer T-cells, to OKT8-positive cells ($T_4/T_8$). On the other hand, Anderson et al reported that no significant difference in the $T_4/T_8$ ratio was observed between the DCM group and control. We studied the ratio of helper/suppressor T-cells using 2 kinds of monoclonal antibodies, Leu-3a and Leu-2a, which recognize T-cell subsets almost identical to those recognized by OKT4 and OKT8. The Leu-3a/Leu-2a ratio for the DCM group was significantly higher than that for the controls, and also very high values were found in 5 of 14 DCM cases (36%). These results were also compatible with those of other investigators.

In this study, we investigated further what kind of abnormal immune-suppression mechanism exists in DCM, with special reference to an impaired IL-2 system. IL-2 is well known to be a lymphokine which can cause T-cells to differentiate and proliferate, and it is deeply involved in the immune
Studies of the changes in IL-2 production in autoimmune diseases such as SLE, rheumatoid arthritis and multiple sclerosis have been reported. Particularly for SLE, changes in IL-2 have been investigated thoroughly using lupus-prone mice (NZB/NZWFl mice etc). It has been shown that IL-2 production and responsiveness to IL-2 of spleen cells are decreased, and that such abnormal changes had existed before the onset of SLE and were accentuated by the development of disease. This suggests the possibility that abnormal IL-2 can be incriminated in the evolution of this autoimmune disease.

In the present study we examined the immunological mechanisms and in particular the proliferative response of PBL, IL-2 production and IL-2R expression in patients with DCM. IL-2 levels and IL-2R expression are regulated by complicated mechanisms and it has not yet been elucidated which system is actually involved in vivo. Roncarolo and his colleagues also investigated the IL-2 system and proliferation in patients with PBL of insulin dependent diabetes mellitus. Their results showed a decrease in IL-2 production, a normal level of the proliferative response of PBL to PHA and normal expression of IL-2R. These results suggest that the normal immune-regulatory system acting through IL-2 may be disturbed in certain disease states. In DCM we observed augmented production of IL-2 in the supernatant of a PBL suspension activated with PHA, although the levels varied widely, suppressed expression of IL-2R and lower responses of PBL proliferation. There are some limitations to our studies. Namely, these studies were not necessarily performed simultaneously and the subjects are not wholly the same patients in the 4 studies. So there are a limited number of patients, mentioned in the results representatively, compared with one another. Recognizing these limitations we interpret our results as indicating that in some patients with DCM, PBL may show an intrinsic defect in the expression of IL-2R and a supplementary increment of IL-2 production with inadequate proliferation of PBL. Although the exact mechanism of these impaired responses of the IL-2 system in DCM and the relation between the immunological disorder and the occurrence of DCM are not yet clarified, such immunological abnormalities may be involved in some patients with DCM. Further study will be needed to clarify these problems.

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REFERENCES


