Lymphocyte Subsets in Patients with Acute Myopericarditis, 
Arrhythmias and Dilated Cardiomyopathy

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To investigate the role of immunoregulatory function in determining the 
clinical course of acute myopericarditis, lymphocyte subsets were analysed 
by laser flow cytometry in 20 patients with acute myopericarditis, 30 with 
various arrhythmias or atrio-ventricular block and 31 with dilated cardio-
myopathy. During the healing stage of acute myopericarditis, patients with 
residual electrocardiographic or left ventricular wall motion abnormalities 
presented altered frequencies of lymphocyte subsets, increased B 1 and re-
duced OKT 8 positive cells with an elevated OKT 4/8 ratio. The abnormal 
pattern was not evident in patients with acute pericarditis nor in those with 
acute myocarditis who recovered completely without residual abnormalities. 
This observation suggested that an imbalance of helper/suppressor T cells 
could modulate the clinical course of acute myopericarditis, either by pro-
ducing extensive and irreversible myocardial damage during acute illness or 
by inducing chronic smoulding myocardial inflammation. Patients with ven-
tricular arrhythmias and left ventricular wall motion abnormalities also 
presented reduced suppressor/cytotoxic T cells, implying that they had been 
suffering from chronic smoulding myocarditis mediated by immunoregulatory 
dysfunction. However, we could not determine whether the imbalance of 
helper/suppressor T cells could mediate the progression from myocarditis 
to dilated cardiomyopathy, since no association was demonstrated between 
the abnormal lymphocyte subsets and mononuclear cell infiltration in endo-
myocardial biopsy sample from patients with dilated cardiomyopathy.

A
cute myocarditis is generally considered to 
be a benign condition which recovers com-
pletely in the majority of patients. However, 
there is a small but significant number of patients 
who suffer from residual myocardial abnormali-
ties, some of which have eventually progressed to 
dilated cardiomyopathy.1-4 Immunoregulatory 
dysfunction has been postulated to mediate the 
progress, since recent studies have described 
abnormal humoral and cellular immune responses 
in patients with dilated cardiomyopathy, in-
cluding deficient suppressor T cell5,6,8-13 or 
natural killer cell function7 and anti-heart anti-
bodies14. Nevertheless, immunological responses 
in patients with acute myocarditis and their 
relationship to the clinical course of the disease 
are poorly understood.

Key words: 
Acute myopericarditis 
Arrhythmias 
Dilated cardiomyopathy 
Lymphocyte subsets 
Regional wall motion abnormality

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In the present study, therefore, we investigated lymphocyte subsets by using laser flow cytometry in patients with acute myopericarditis, various arrhythmias or atrio-ventricular block and dilated cardiomyopathy. We then discussed whether an imbalance in helper/suppressor T cell function modulates the severity of acute myocarditis, or whether it induces chronic smoulding myocardial inflammation which ultimately progresses to dilated cardiomyopathy.

SUBJECTS AND METHODS

Study patients

Four groups of patients were studied. The control group included 62 healthy volunteers, 46 males and 16 females, with an average age of 47 ± 12 years. The acute myopericarditis group included 20 patients, 8 males and 12 females, with an average age of 41 ± 14 years. The diagnosis of acute myopericarditis was established when they met the following criteria; 1) gradually changing patterns of ST-segment and T wave in serial electrocardiograms, 2) preceding flu-like syndrome followed by cardiac symptoms like chest pain, dyspnea or syncope, and 3) laboratory signs of acute inflammation indicated by elevated erythrocyte sedimentation rate, increased white blood cell count or positive C-reactive protein. Twelve patients who additionally demonstrated elevations in CK and GOT, abnormal left ventricular wall motion, evolution of abnormal Q wave or conduction disturbances were diagnosed as having acute myocarditis. Eight patients lacking these signs of myocardial involvement were defined as having pericarditis. The third group included 18 patients with ventricular arrhythmias, 8 males and 10 females with a mean age of 46 ± 14 years. Clinical examinations including cardiac catheterization, coronary angiography and endomyocardial biopsy revealed no underlying diseases in these patients. This group included 8 patients with ventricular tachycardia. The fourth group included 12 patients, 11 males and 1 female with a mean age of 44 ± 12 years, with supraventricular arrhythmias (4 with atrial fibrillation, 3 with paroxysmal supraventricular tachycardia) or atrio-ventricular block (5 patients). As in the previous group, no underlying causes were found in these patients after thorough examinations. The fifth group included 31 patients, 24 males and 7 females with an average age of 49 ± 12 years, with dilated cardiomyopathy who underwent endomyocardial biopsy. Dilated cardiomyopathy was defined by a dilated left ventricle (end-diastolic diameter of the left ventricle > 55 mm) and impaired left ventricular wall motion (fractional shortening < 30%). Patients with any evidence of systemic hypertension, coronary artery disease, valvular heart disease, congenital heart disease, hypertrophic cardiomyopathy or other cardiac or systemic diseases involving the heart muscle were excluded from this group.

Evaluation of left ventricular regional wall motion and mononuclear cell infiltration

Residual regional wall motion abnormalities in the healing stage of acute myopericarditis were assessed by left ventriculography in 10 patients, and by 2-D echocardiography in the remaining 10 patients, which were performed 3 or more months after the onset of cardiac symptoms. Regional wall motion was again determined by left ventriculography in all patients with ventricular or supraventricular arrhythmias or atrio-ventricular block. Three independent and experienced observers evaluated the regional wall motion in the left ventriculogram or 2-D echocardiogram. Endomyocardial biopsy was performed in 8 patients with acute myopericarditis and in all patients with dilated cardiomyopathy. Two or three samples were obtained from the right side of the ventricular septum. Mononuclear cell infiltration was taken as positive when more than 5 cells were observed in the high power field (250 x 250).

Lymphocyte subsets assays

Peripheral venous blood was collected into a heparinized tube and 100 µl of whole blood was mixed with 100 µl of FITC-conjugated monoclonal antibody; OKT 4 (helper/inducer cells), OKT 8 (suppressor/cytotoxic cells), OKT 11 (pan T cells): (Ortho Diagnostic Systems), and B 1 (pan B cells): (Coulter Immunology). The mixture was placed at 4°C for 30 min and then hemolysed by adding 3 ml of a lysing agent (NH₄Cl 8.26 g/L, KHCO₃ 1.0 g/L, EDTA-4Na 0.0037 g/L). After centrifugation at 300g for 10 min, the pellet was washed twice by phosphate buffer saline (PBS) and resuspended in 2 ml of PBS. Fluorescence analysis was performed by laser flow cytometry (Ortho Diagnostic Systems). Approximately 2,000 lymphocytes were selected and the fraction of lymphocytes binding to a particular monoclonal antibody was determined by comparing the number of fluorescing cells...
to the total lymphocyte population.

In patients with acute myopericarditis, the lymphocyte subsets were determined during the healing stage from blood samples obtained 3 months or more after the onset of illness and were used for comparison. Serial changes in the subsets were additionally investigated in 6 patients with acute myopericarditis during the acute and convalescent stages.

**Statistical analysis**

Values are presented as means ± standard deviation. Comparisons of frequencies of the lymphocyte subsets between control subjects and each patient group or between 2 subgroups of patients were performed by an unpaired student's t test. Probability values of less than 0.05 were considered statistically significant. The normal range of each subset was defined as a 95 percent confidence interval of the control subjects.

**RESULTS**

Table I presents frequencies of lymphocyte subsets in the five study groups. In the peripheral blood of patients in the healing stage of acute myopericarditis, 12 patients with acute myocarditis showed significantly reduced frequencies of OKT 11 and OKT 8 positive cells with a significantly higher OKT 4/8 ratio, as compared with control subjects. On the other hand, there were no differences in frequencies of the lymphocyte subsets between patients with acute pericarditis and the control group. In follow-up examinations 3 or more months after the onset, all patients with acute pericarditis and 6 of those with acute myocarditis were found to have recovered completely without any evidence of residual abnormalities. The remaining 6 patients with acute myocarditis showed residual regional wall motion abnormalities of the left ventricle (5 patients), and persistent abnormal Q-wave (1 patients). Four patients showed flat or negative T waves. These patients with acute myocarditis and incomplete recovery showed significantly higher frequencies of B 1 positive cells and lower frequencies of OKT 8 positive cells with an increased OKT 4/8 ratio, as compared with those.

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**Table I**

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>Monoclonal Antibody (% Cells Fluorescing)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B 1</td>
</tr>
<tr>
<td>Control subjects</td>
<td>62</td>
</tr>
<tr>
<td>Acute myopericarditis</td>
<td>20</td>
</tr>
<tr>
<td>Pericarditis</td>
<td>8</td>
</tr>
<tr>
<td>Myocarditis</td>
<td>12</td>
</tr>
<tr>
<td>Complete recovery</td>
<td>14</td>
</tr>
<tr>
<td>Incomplete recovery</td>
<td>6</td>
</tr>
<tr>
<td>Ventricular arrhythmias</td>
<td>18</td>
</tr>
<tr>
<td>Asynery (-)</td>
<td>7</td>
</tr>
<tr>
<td>Asynery (+)</td>
<td>11</td>
</tr>
<tr>
<td>SVA or A-V block</td>
<td>12</td>
</tr>
<tr>
<td>SVA</td>
<td>7</td>
</tr>
<tr>
<td>A-V block</td>
<td>5</td>
</tr>
<tr>
<td>Dilated cardiomyopathy</td>
<td>31</td>
</tr>
<tr>
<td>Cell infiltration (-)</td>
<td>24</td>
</tr>
<tr>
<td>Cell infiltration (+)</td>
<td>7</td>
</tr>
</tbody>
</table>

Values are means ± SD.

*p < 0.05, **p < 0.01, as compared with controls.

++p < 0.05, ++p < 0.01, as compared between subgroups.

SVA = supraventricular arrhythmias.
Fig. 1. Lymphocyte subsets in patients with acute myocarditis (closed circle) and acute pericarditis (open circle) in the healing stage. Patients with acute myocarditis who were suffering from residual electrocardiographic abnormalities or impaired left ventricular wall motion, indicated by recovery (−), present increased frequencies of B 1 and reduced frequencies of OKT 8 cells with an elevated OKT 4/8 ratio. Shaded areas show 95% confidence intervals of the subsets in control subjects.

of either control subjects or patients with acute myopericarditis who had recovered completely. No abnormalities in frequencies of lymphocyte subsets were evident in patients with complete recovery, except in one patient with acute myocarditis (Fig. 1). Fig. 2 shows serial changes in the lymphocyte subsets during the acute and convalescent stages of myopericarditis. Gradual increases in frequencies of OKT 11, OKT 4 and OKT 8 positive cells were observed, while B 1 positive cells tended to decrease, along with subsiding myocardial inflammation. The OKT 4/8 ratio was variable from patient to patient and showed no consistent change during these stages.

In patients with ventricular arrhythmias with no evidence of underlying causes, frequencies of OKT 4 positive cells were significantly higher and those of OKT 8 positive cells were lower with a significantly higher OKT 4/8 ratio as a group, as compared with control subjects. These abnormal frequencies of the lymphocyte subsets were more evident in patients with regional wall
Fig. 2. Serial changes in the lymphocyte subsets during the acute and convalescent stages of myopericarditis. During the acute stage within 10 days after onset of cardiac symptoms, there is a reduction of OKT 11 cells and an elevation of B cells, which return to normal levels. OKT 4 and OKT 8 positive cells show gradual increases in these stages, while no consistent serial change is observed in the OKT 4/8 ratio. Shaded areas indicate 95% confidence intervals of the subsets in control subjects.

motion abnormalities. In the group of supraventricular arrhythmias or atrio-ventricular block patients, there was a slight but significant decrease in OKT 8 positive cells with an increased OKT 4/8 ratio. However, no appreciable differences in the lymphocyte subsets were observed between patients with supraventricular arrhythmias and those with atrio-ventricular block or between those with left ventricular regional wall motion abnormalities and those without. Patients with dilated cardiomyopathy also demonstrated significantly higher frequencies of OKT 4 positive cells and lower OKT 8 positive cells with a resultant increase in the OKT 4/8 ratio. However, there was no relationship between frequencies of the lymphocyte subsets and the presence or absence of mononuclear cell infiltration in biopsy specimens.

DISCUSSION

Lymphocyte subsets in acute myopericarditis

The present study demonstrated that during the healing stage of patients with acute myo-
carditis, incomplete recovery was associated with abnormal lymphocyte subsets in the peripheral blood, specifically, increased frequencies of B1 and decreased frequencies of OKT S positive cells with a higher OKT 4/8 ratio. These abnormal patterns of the lymphocyte subsets were not observed in patients with acute pericarditis or in those acute myocarditis who had recovered completely without any evidence of residual electrocardiographic or regional wall motion abnormalities. It was, thus, of note that an imbalance of helper-suppressor T cells associated with an increase in B cells was evident in the particular subgroup of patients with acute myopericarditis. Suppressor T cell function can either be impaired due to a decrease in frequency of the cells, a reduction of suppressor activity of the cells or by both. Previous reports have yielded conflicting results on suppressor T cell function in patients with myopericarditis. Whereas Kishidze observed reduced frequencies of suppressor T cells in the peripheral blood of patients with myocarditis, Maisch and Kishimoto et al could not confirm the observation. In our previous study, we were also unable to find an abnormal pattern of the lymphocyte subsets in the healing stage of acute myopericarditis. In investigations of concanavalin-A or phytohemagglutinin stimulated suppressor T cell activity, Eckstein et al and Kishimoto et al described a significant reduction of suppressor activity in patients with myocarditis. However, Maisch and Goch et al failed to demonstrate the abnormality. Possible explanations for this discrepancy may include different methods used for assessing suppressor T cell function, patients at different stages of the disease or different healthy controls. On the other hand, the present study indicated that an abnormal pattern of lymphocyte subsets was not consistently observed in all patients with acute myopericarditis, but was only evident in the specific subgroup of patients associated with incomplete recovery. Therefore, a different population of study patients could be an alternative explanation for inconsistent results for suppressor T cell function in previous reports.

To further investigate whether or not the abnormal pattern of lymphocyte subsets is induced by viral infection, we studied serial changes in the lymphocyte subsets during the acute and convalescent stages of acute myopericarditis. In the acute stage within 10 days after onset, we observed a reduction of pan T cells and an increase in B cells which gradually returned to normal levels. The findings suggested that viral infection initiated B cell proliferation, leading to the production of anti-viral antibodies. Frequencies of helper/inducer and suppressor/cytotoxic T cells gradually increased during convalescence along with pan T cells. These changes, however, were much smaller than variabilities between patients and these was no consistent serial trend in the helper/suppressor T cell ratio. Therefore, it seemed unlikely that viral infection induced a significant alteration in the immunoregulatory function. This observation, in turn, implied that the imbalance between helper/suppressor T cells observed in patients with acute myocarditis and incomplete recovery could be an inherent defect rather than a virus-mediated abnormality.

**Lymphocyte subsets in arrhythmias and block**

Experimental and clinical studies have demonstrated that ventricular and supraventricular arrhythmias and conduction disturbances frequently occur in the chronic stage of myocarditis. Recent studies using endomyocardial biopsy have shown that patients with clinically silent myocarditis are included in those with otherwise unexplained ventricular arrhythmias. Regional wall motion abnormalities have been described to be another manifestation of residual myocardial damage after acute myocarditis. Hoshino et al proposed a causal relationship between ventricular aneurysm and arrhythmias in Syrian golden hamsters infected with Coxsackie B-1 virus. In the present study, we also noted that regional wall motion abnormalities of the left ventricle were the most frequent manifestation of incomplete recovery from acute myocarditis, being present even in those having no electrocardiographic abnormalities. We therefore postulated that ventricular arrhythmias in association with left ventricular wall motion abnormalities could be a possible manifestation of myocarditis. An interesting finding in this regard is that abnormal frequencies of helper/inducer and suppressor/cytotoxic T cells in patients with ventricular arrhythmias were associated with the presence of the regional wall motion abnormalities. This observation could raise the possibility that these patients with ventricular arrhythmias and wall motion abnormalities had been suffering from chronic smouldering myocarditis which was mediated by immunoregulatory defects.
Patients who showed supraventricular arrhythmias or atrio-ventricular block of unexplained etiology presented a slight but significant increase in the OKT 4/8 ratio. Although the small number of study patients did not allow us to perform further analyses, it is conceivable that some patients with these arrhythmias or block could be related to smoulding myocarditis associated with immunoregulatory dysfunction.

**Lymphocyte subsets in dilated cardiomyopathy**

Viral myocarditis has long been postulated to be one of the etiologies of dilated cardiomyopathy. Recent studies have demonstrated abnormal humoral, cellular and immunoregulatory responses in patients with dilated cardiomyopathy that could produce a continuing low grade inflammatory response and ultimately culminate in ventricular dysfunction and dilatation. Among these, deficient function of suppressor T cells has been the focus of increasing attention but still remains controversial. These conflicting results could be at least partly explained by the difference in study population, since dilated cardiomyopathy is now considered to be the terminal stage of etiologically different diseases. We then divided the study patients with dilated cardiomyopathy into those with and those without mononuclear cell infiltration on endomyocardial biopsy. Nonetheless, we failed to find any difference in the lymphocyte subsets between the two groups, although patients with dilated cardiomyopathy as a group presented reduced frequencies of suppressor/cytotoxic T cells. Possible explanations for this lack of relation may include: 1) a limited and insufficient pieces of biopsy samples, 2) ill-defined criteria for biopsy diagnosis of residual myocardial inflammation or 3) involvement of other immunological defects, rather than deficient suppressor function, in the pathogenesis of myocarditis-related dilated cardiomyopathy.

**Possible role of imbalance of helper/suppressor T cells in the development of myocardial damage**

Previous experimental studies on viral myocarditis have demonstrated that immunological responses play an important role in the pathogenesis of myocardial damage. They have focused on the role of cytotoxic T cells capable of lysing virus-infected myocardial cells and producing myocardial inflammation. On the other hand, several studies have demonstrated circulating heart-reactive antibodies in myocarditis. Recent reports by Maisch et al. re-emphasized the importance of humorally-mediated myofiber cytotoxicity. They described that muscle-specific anti-myolemmal and anti-sarcolemmal antibodies can be detected 12 to 14 days after the onset of acute myocarditis as well as in patients with dilated cardiomyopathy. These antibodies could induce cell death either by complement-activated antibody-mediated cytolysis or by antibody-dependent cellular cytotoxicity (ADCC), while Maisch suggested the former possibility in myocarditis.

Defective suppressor T cell function, either by reduced number or by impaired activity, could enhance helper T cell proliferation and induce effector cytotoxic T cells and B lymphocyte clones to produce anti-heart antibodies. The imbalance between helper/suppressor T cells, thus, can induce excessive immune reactions and produce further pathological damages, as demonstrated in several autoimmune diseases. In the present study, it is also conceivable that patients with acute myocarditis who had the imbalance could have developed excessive immune reactions and produced residual myocardial damage. On the other hand, in patients with a normal pattern of lymphocyte subsets, virus-induced immune reactions might have been self-limited, leading to complete recovery. However, we could not determine whether defective suppressor T cell function produced extensive and irreversible immune-mediated myocardial damage in the acute stage of illness, or induced persistent smoulding inflammation progressing to chronic myocarditis. The absence of abnormal lymphocyte subsets in patients with isolated pericarditis may suggest that immunoregulatory function could determine the severity of acute illness. On the other hand, increased B cells in patients with myocarditis and incomplete recovery implied that the imbalance of helper/suppressor T cells was providing a persistent stimulus, even in the healing stage, to B cell proliferation and antibody production, thus resulting in smoulding inflammation and residual myocardial damage. The latter speculation that immunoregulatory dysfunction is related to persisting chronic myocarditis is further supported by the observation in patients with ventricular arrhythmias that the imbalance of helper/suppressor T cell was mainly found in those with left ventricular wall motion abnormalities. The lack of significant correlation between the immunoregulatory defect and mono-
nuclear cell infiltration in patients with dilated cardiomyopathy was somewhat conflicting to these considerations. Poor accuracy of the biopsy findings in diagnosing residual myocardial inflammation could be a possible explanation. Alternatively, we could consider that immunological defects other than lymphocyte subtypes may be operative in the pathogenesis of myocarditis-related dilated cardiomyopathy.

Finally, it should be noted that the present study investigated merely a part of the sophisticated immunological cascade. More comprehensive studies on the immunological system both in the peripheral blood and in the myocardium in situ will be required to understand the exact role of immunoregulatory function in determining the severity of acute myocardial damage after viral infection, or in inducing persistent smouldering inflammation which may eventually progress to dilated cardiomyopathy.

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