LYMPHATIC SUBPOPULATIONS AND THEIR TRANSITION IN
MYOCARDIAL TISSUE AND PERIPHERAL BLOOD OF
PATIENTS WITH BIOPSY-PROVEN MYOCARDITIS

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To determine abnormal immune regulation in biopsy-proven active and healed myocarditis cases, lymphatic subpopulations in myocardial tissue and peripheral blood were studied. Among 53 cases examined, 19 were active myocarditis (M) and 34 were healing or healed (HM). Five cases of myocarditis were studied sequentially. The percentages of pan T-cells, B-cells, helper/inducer T-cells (Th/i), suppressor/cytotoxic T-cells (Ts/c), etc per total marker positive cells were calculated by use of monoclonal antibodies. In myocardial tissue, the percentage of Th/i was significantly lower in HM than M (p < 0.01). The helper/suppressor ratio (OKT4/8) in peripheral blood was 2.43 ± 0.43 (mean ± SE) in M, 1.61 ± 0.19 in HM and 1.34 ± 0.12 in age-matched controls. In 5 progressive studied cases of M, there was a decrease of the helper/suppressor ratio at 1 to 6 months after the myocarditis. It was concluded that subsidence of the immune reaction in myocardium is related to the healing process of myocarditis and may suggest improved prognosis.

RECENTLY, with increased use of endomyocardial biopsy, many patients with presumptive diagnosis of dilated cardiomyopathy (DCM) or various arrhythmias have been found to have active lymphocytic myocarditis1-3 or high incidence of chronic inflammation4. Several studies of the subpopulations of inflammatory infiltrates in situ have demonstrated immunological abnormalities, especially due to cell-mediated tissue injury, in myocarditis or DCM.5-13 However, the proportions of these subpopulations were uncertain and relations between the subpopulations and inflammation in tissue is still obscure. The imbalance between helper and suppressor T cells in peripheral blood, especially due to the decrease of suppressor T cells, in patients with DCM has been reported14-16 Abnormality of autoimmunological mechanism may cause myocardial injury. So far, several studies of immunological abnormalities have been reported, but no studies of lymphatic subpopulation in situ during transition have yet been reported.

The aim of the present study was to determine, through the use of monoclonal antibodies, any differences that might exist in lymphatic subpopulations in myocardial tissue and peripheral blood of patients with biopsy-proven active and healed myocarditis.

Key words:
Myocarditis
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TABLE I DIAGNOSTIC CRITERIA FOR ACTIVE AND HEALED MYOCARDITIS IN ENDOMYOCARDIAL BIOPSY

<Active myocarditis>
1) Myocyte injury
2) Inflammatory cellular infiltration; > 5 cells/HMF
3) Interstitial edema

<Healed myocarditis>
(Reliable diagnosis)
1) Subsequent follow-up course from acute phase

(Suggestive diagnosis)
1) Irregular focal interstitial fibrosis
2) Newborn small blood vessels
3) Slight degree of inflammatory cellular infiltration
4) Regenerative-like change of myocyte
   (hypertrophy, double nuclei, etc.)

and dysrhythmias, endomyocardial biopsy was performed at Showa University Hospital from May, 1986 to March, 1988. Fifty-three patients were diagnosed as having either active myocarditis (M), or healing or healed myocarditis (HM). Diagnosis of M and HM was based on the findings listed in Table I (Fig. 1A, B). These 53 (30 men and 23 women ranging in age from 13-75) were subjects for the present study. Nineteen had M and 34 had HM (reliable, 12; suggestive, 22). Mean ages were 57.1 years for M patients and 42.5 years for HM patients. The chief complaints in 53 cases are listed in Table II. Various electrocardiographic abnormalities, chest pain or oppression and palpitation were the most common complaints. Two or three specimens from the right or left ventricle were obtained by endomyocardial biopsy, using Machida's Biopette. Studies were carried out during the progress of 5 cases of myocarditis. Only 10 of the patients were suspected of myocarditis prior to biopsy.

Fig.1 A: Active myocarditis; disappearance, melting and eosinophil degeneration of myocytes with inflammatory cellular infiltration (Hematoxylin-eosin stain, x200).
B: Healed myocarditis; hypertrophy and disarray of myocytes, irregular interstitial fibrosis and slight cellular infiltration (a case of sequential examination, Azan-Mallory stain, x200).
C: Leu4 (+) cells (Immunoperoxidase stain, x200).
D: OKT8 (+) cells (Immunoperoxidase stain, x400).

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Various electrocardiographic abnormalities were documented in 73.6% of the patients (39 cases) (Table III). Out of 19 patients in the M group, evidence of sick sinus syndrome was seen in 10 and various supraventricular arrhythmias were observed. In the HM group (34 cases), paroxysmal supraventricular tachycardia (PSVT) and AV block were common. Twenty-three patients of the HM group had clinical arrhythmia and others had hypertrophic cardiomyopathy, chest pain syndrome, etc. Of the cardiac function in 34 cases, 4 (M, 2; HM, 2) showed reduction in both ejection fraction (<60.0%) and cardiac index (<3.0 L/min/m²). Whereas cardiac function of 21 was normal. In most of the 53 cases, inflammatory findings in blood examination were negative.

The biopsied specimens were fixed in 10% formalin, embedded in paraffin and examined by hematoxylin-eosin stain, Azan-Mallory stain, etc. Frozen 6 μm sections from unfixed specimens were sliced with a mirror section technique to obtain two equal cut surfaces, after which they were fixed with acetone. We then performed immunoperoxidase staining using the avidin-biotin-peroxidase complex method (ABC method); 3, 3'-diaminobenzidine-tetrahydrochloride was utilized as the chromogen (Fig. 1C, D).

Monoclonal antibodies used in this study were: DAKO-LC (Dakopatts), leucocyte common antigen; Leu4 (Becton Dickinson), pan T-cells; Leu 12, B-cells; OKT4 (Ortho Diagnostic System), helper/inducer T-cells (Th/1); OKT8, suppressor/cytotoxic T-cells (Ts/c); Leu 11, natural killer killer cells; and LeuM3, monocytes/macrophages. A control slide (without monoclonal antibodies) was used to determine background staining. We

<table>
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<tr>
<th>TABLE III ELECTROCARDIOGRAPHIC FINDINGS (53 CASES)</th>
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<tr>
<td>Finding</td>
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<tr>
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<tr>
<td>Sinus brady. or sinus arrest</td>
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<tr>
<td>Atrial fibrillation</td>
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<td>ST-T abnormality</td>
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<tr>
<td>VPC</td>
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<tr>
<td>AV block (more than IInd degree)</td>
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<td>VT</td>
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<tr>
<td>Bundle branch block</td>
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<tr>
<td>AV functional rhythm</td>
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<td>PSVT</td>
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<tr>
<td>Others</td>
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Fig.2. Subpopulations of inflammatory infiltrates. In the M group, the total marker (+) cells per HMF were 1.8 times as many as those of the HM group. The number of helper/inducer cells was small in the HM group (p < 0.01).

counted the number of marker positive cells in a highly magnified field (HMF) in each case, totaled them, and calculated the proportion of each inflammatory infiltrate to the total number of marker positive cells. The total number of marker positive cells and the proportions were averaged in the M and HM groups. We computed the ratios of OKT4/Leu4 and OKT8/Leu4 in the common cut surface obtained by the mirror section technique, and compared M with HM by $\chi^2$ analysis.

We examined the helper/suppressor ratio (OKT4/8) in peripheral blood of 14 cases of M, 15 cases of HM and 14 age-matched controls. The data were statistically analyzed by unpaired Student's t test.

RESULTS

The total number of marker positive cells and the percentages of subpopulations of inflammatory infiltrates in the M and HM groups are shown in Fig. 2. In the M group, the total marker positive cells per HMF were 1.8 times those in the HM group. The number of Th/i was small in the HM group compared to that in the M group ($p < 0.01$). The number of B-cells was slightly small in the HM group and no remarkable change was found in the numbers of

Fig.3. The OKT4/OKT8 ratio in peripheral blood. A high OKT4/OKT8 ratio was present in the M group compared with the ratios of the HM group ($p < 0.05$) and of the normal control groups ($p < 0.01$).

Fig.4. Transition of OKT4/OKT8 ratio in peripheral blood of 5 cases of the M group. Decrease of the OKT4/OKT8 ratio was demonstrated in progressive examination.

Tc, monocytes/macrophages or natural killer/killer cells.

The helper/suppressor ratios in peripheral blood were $2.43 \pm 0.34$ in the M group, $1.62 \pm 0.19$ in the HM group and $1.34 \pm 0.12$ in the control group (mean $\pm$ S.E.). Individual values are shown in Fig. 3.

In progressive data of 5 cases of the M group, the high helper/suppressor ratio was decreased 1 to 6 months after the first examination. Individual values are shown in Fig. 4.

DISCUSSION

Some investigators have recently utilized monoclonal antibodies to analyze infiltrating mononuclear cells in cardiac tissue of myocarditis or DCM. Marboe et al examined 20 cases of biopsy-proven myocarditis where they noted 60–90% of the T cells of the OKT8 (+) subset. Hammond et al reported that 50% of the infiltrating cells indicated macrophages, 30% were T lymphocytes, and approximately half of these T cells were OKT8 (+). In experimental murine myocarditis, T cells accounted for approximately 80% of the cells in the myocardium on days 7 and 14 after virus inoculation. Deguchi et al reported that infiltration of lymphocytes was predominant on day 14 after virus inoculation, and the T cell percentage was the highest ($16.4 \pm 2.6\%$) in the total interstitial cell population. The results of these studies and our study almost
corresponded with regard to the high percentage of T lymphocytes in the infiltrating cells. In
our study, the T cell percentage was also predominant (55.8%) among the subpopulations
of inflammatory infiltrates in the M group. In the
HM group, the percentage of T cell was about the same (50.0%). These observations suggest that
T lymphocytes may be significant in myocarditis or DCM. Differences in the percentages of T cell
in different studies may be due to the stage and severity of myocarditis.

In uninflamed and nondilated hearts, most of
the interstitial and mononuclear cells expressed
T cell markers, with Th/i being slightly more
prominent than Ts/c (the OKT4/OKT8 ratio was
1.44)17 Cassling et al observed that in patients
with active myocarditis the largest subset of cells
was the Ts/c population with a resultant OKT4/
OKT8 ratio of 0.30 coupled with a marked
absolute minority in the macrophage/monocyte
series.9 The elevation in OKT8 marked T cells
agrees with the data of Zee-Cheng et al2 and
Marboe et al10 These observations correspond to
those in experimental murine myocarditis11–13
In our study, the OKT4/OKT8 ratio was 0.61 in
the M group and 0.13 in the HM group; most T
cells were OKT8 (+). The role of these OKT8 (+)
cells remains obscure, but it is postulated that
they may invade and destroy nonnecrotic
muscle fibers in some inflammatory myopathies,
and the autolysing cells are considered to be
cytotoxic rather than suppressor cells.18 These
OKT8 (+) cells may be responsible for the lesions
seen in myocarditis, but many OKT8 marked T
cells were also observed in the HM group. This
does not necessarily imply injury of the myocar
dium by Ts/c.

In the HM group, there were markedly fewer
OKT4 (+) cells than in the M group. The role of
Th/i is to aid in the production of antibodies or
in cell-mediated immune responses. In active
myocarditis, Th/i may be activated, and thus
enhance the immune response and aid reduction
of the myocardial microenvironment. In healed
myocarditis, Th/i may be decreased in corre
spondence with the subsidence of myocardial
inflammation. This suggests that Th/i may
deck on the activity of myocarditis.

Conversely, an increased OKT4/OKT8 ratio
was found in peripheral blood of many patients
who suffered from some autoimmune diseases.19
However, the OKT4/OKT8 ratio in the tissue was
controversial. In acute and chronic B virus
hepatitis, acquired low globulinenia, bone

marrow transplantation, etc., decreased OKT4/
OKT8 ratio in peripheral blood has been re
ported20,21 Some patients with idiopathic
dilated cardiomyopathy had a helper/suppressor
cell ratio higher than normal, and the percentage
of Th/i was significantly greater.14,15 On the
other hand, Franceschini et al reported that the
percentage of peripheral blood lymphocytes with
suppressor/cytotoxic activity was reduced in
patients with dilated cardiomyopathy when
compared to normal subjects.16 Anderson et al
reported that relative percentages of lymphocyte
subsets and the helper/suppressor ratio were not
consistently changed in patients with dilated
cardiomyopathy.22

In the present study, a high helper/suppressor
ratio was found in the peripheral blood of the
group of biopsy-proven myocarditis, when com
pared to the ratios in the group with healing or
healed myocarditis (p < 0.05) and the normal
control group (p < 0.01). In progressive studies,
the helper/suppressor ratio in peripheral blood
was decreased in the healing stage.

It was concluded that subsidence of the
immune reaction in myocarditis is related to the
healing process of myocarditis and may suggest
improved prognosis.

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