A CASE OF MYOCARDITIS WITH IMMUNOLOGICAL IDENTIFICATION OF MYOCARDIAL AND PERIPHERAL LYMPHOCYTE SUBSETS

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Immunological identification of lymphocyte subsets in a patient with myocarditis revealed an increase in myocardial OKT 11 (pan T), OKT 4 (inducer/helper T) and OKT 8 (suppressor/cytotoxic T) subsets associated with a transient decrease in the percentage of circulating OKT 3 (pan T) and OKT 4 (inducer/helper T) subsets. This decrease may be explained by the accumulation of these subsets in the diseased myocardium. Specific antigenic markers on lymphocytes at the site of myocardial inflammation in the acute stage of myocarditis differ from those on corresponding peripheral lymphocytes. This observation may highlight the immuno-pathogenetic mechanisms involved in the development of myocarditis.

THE importance of immune dysfunction in myocarditis has been postulated1–3 but remains to be demonstrated. In this study, we describe the serial changes in lymphocyte subsets in the peripheral blood, and the immunohistological findings of biopsy specimens, in a patient with myocarditis of unknown origin. The possible role of the infiltrating T-cell subsets in the development of myocarditis and theories about its pathogenesis are discussed.

PATIENT PROFILE
The patient was a 15-year-old girl with myocarditis of unknown origin.
She was admitted on February 28, 1985

because of chest pain. She had had nausea, vomiting and general malaise a week before admission. The diagnosis of myocarditis was established on the basis of compatible electrocardiograms (complete AV block and ST segment elevation), normal coronary arteriograms during cardiac catheterization, and laboratory findings (elevated erythrocyte sedimentation rate, creatine phosphokinase, glutamine ornitine transferase and lactic dehydrogenase).

There were no significant changes in any virus titers in paired sera during the second week of convalescence or identification of possible agents for myocarditis. However, the titer of para-influenza 3 fluctuated. Thus, viral myocarditis was suspected.

Cardiac catheterization with right-sided endomyocardial biopsy was performed on March 7 and again on April 4 (Fig. 1); left ventriculograms showed moderately decreased left ventricular wall motion (Fig. 1). Biopsied samples were processed for immunohistologic studies and pe-
Peripheral lymphocyte subsets were analyzed serially.

METHODS

Peripheral Lymphocyte Subsets:
Peripheral blood collected in heparin (20 ml) was washed twice with Hanks' balanced salt solution and the red cells lysed by hypotonic shock. The lymphocyte fraction was obtained by the Ficoll-Hypaque gradient method and the lymphocytes counted in a standard hemocytometer. The cells were finally suspended at a concentration of 1 x 10^6 cells/ml in RPMI-1640 media with 2.5% fetal calf serum and 0.1% NaN₃. T-cell subsets were stained by using the commercially available fluorescein isothiocyanate (FITC)-labeled monoclonal antibodies (Ortho-mune, Ortho Diagnostic System K.K. and Becton Dickinson, Becton Dickinson Monoclonal Center, Inc.): OKT 3 (770310), pan T-cell marker; OKT 4 (770410), inducer/helper T-cell marker; OKT 8 (770810), suppressor/cytotoxic T-cell marker; OK Ia (770220), B cell, activated T-cell and monocyte marker; OKB 7 (771720), B-cell marker; Leu 7 (7393), natural killer/killer cell marker; and Leu 11 (7523), natural killer cell marker. These cell preparations were analyzed by laser flow cytometry (Ortho Spectrum III).

Immunoperoxidase Staining of Endomyocardial Samples:
Four-microsections were cut from the frozen blocks of the biopsy sample on a cryostat at -20°C, placed on glass slides, and fixed in 95% cold methanol. Cell surface markers were demonstrated in situ by 3-ami-no-9-ethyl-carbazole immunoperoxidase staining, using the commercially available monoclonal antibodies as the first layer (Ortho-mune, Ortho Diagnostic System K.K.): OKT 11 (780110), pan T-cell marker; OKT 4 (780040), inducer/helper T-cell marker; and OKT 8 (780080), suppressor/cytotoxic T-cell marker. Peroxidase conjugated goat anti-mouse immunoglobulin was used as the second layer of antibodies.

RESULTS
Table I summarizes the serial changes in the
TABLE 1 SERIAL CHANGES OF LYMPHOCYTE SUBSETS (%) IN THE PERIPHERAL BLOOD OF THE PATIENT WITH MYOCARDITIS

<table>
<thead>
<tr>
<th>Days</th>
<th>Mar. 2 (7*)</th>
<th>Mar. 4 (9*)</th>
<th>Mar. 26 (31*)</th>
<th>Apr. 4 (40*)</th>
<th>Jul. 20 (147*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OKT 3</td>
<td>(70.2 ± 2.3)</td>
<td>55.3</td>
<td>68.6</td>
<td>53.1</td>
<td>67.3</td>
</tr>
<tr>
<td>OKT 4</td>
<td>(38.8 ± 6.0)</td>
<td>21.0</td>
<td>38.6</td>
<td>16.7</td>
<td>31.1</td>
</tr>
<tr>
<td>OKT 8</td>
<td>(26.3 ± 4.1)</td>
<td>33.5</td>
<td>29.6</td>
<td>34.4</td>
<td>34.9</td>
</tr>
<tr>
<td>OKT 4/8</td>
<td>(1.5 ± 0.4)</td>
<td>0.6</td>
<td>1.3</td>
<td>0.5</td>
<td>0.9</td>
</tr>
<tr>
<td>OK Ial</td>
<td>(11.8 ± 2.0)</td>
<td>24.4</td>
<td>-----</td>
<td>15.0</td>
<td>22.1</td>
</tr>
<tr>
<td>OKB 7</td>
<td>(10.5 ± 2.7)</td>
<td>14.3</td>
<td>9.8</td>
<td>-----</td>
<td>5.7</td>
</tr>
<tr>
<td>Leu 7</td>
<td>(13.2 ± 2.6)</td>
<td>1.0</td>
<td>6.7</td>
<td>9.8</td>
<td>24.2</td>
</tr>
<tr>
<td>Leu 11</td>
<td>(14.7 ± 2.6)</td>
<td>1.2</td>
<td>-----</td>
<td>6.5</td>
<td>18.5</td>
</tr>
</tbody>
</table>

*On the assumption of history taking.

( ) ----- age-matched normal ranges in our laboratory (n = 5)

Fig.2. Histological sections (March 7, 1985) of the biopsied myocardium of the right ventricle. Marked cellular infiltration is evident.

(A) = Hematoxylin-eosin stain,
(B) = Hematoxylin-eosin stain,
(C) = OKT 4 positive stain,
(D) = OKT 11 and OKT 8 positive stains.

(A) = x18, (B) = x 180, (C) = x 450 (540),
(D) = x 370

Peripheral lymphocyte subsets. In general, OKT 3 and OKT 4 were moderately decreased in the acute stage, but returned to almost normal.

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Fig. 3. Histological sections (April 4, 1985) of the biopsied myocardium of the right ventricle. Marked myocardial fibrosis is shown. OKT 11 positive cells (arrow heads) are still present. (A) = Hematoxylin-eosin stain; (B) = OKT 11 positive stain. (A) = × 450, (B) = × 450.

levels on April 4; OKT 8 did not change markedly. OK T-1 increased, and Leu 7 and Leu 11 decreased markedly in the acute stage of the illness.

The immunohistological study of the biopsy samples revealed that most of the stained cells, OKT 11 (30–40%), OKT 4 (20–30%) and OKT 8 (20%), were positive on March 7 (Fig. 2). A few OKT 11 positive cells (10–15%) were found on April 4 (Fig. 3); OKT 4 and OKT 8 positive cells were few (0–5%). There were no distinct changes in anatomic distribution of the lymphocyte subsets.

DISCUSSION

Pathogenesis of Myocarditis:
The etiology, pathogenesis and natural history of human myocarditis are poorly understood. The animal studies reported to data have described certain immunologic events in peripheral blood or spleen cells and have indirectly defined the inflammatory cell populations which infiltrate the myocardium. However, identification of cell types present at the actual site of immunologically mediated damage is essential for a complete understanding of pathogenesis in diseases in which lymphocyte subset changes in the peripheral blood may not reflect altered immunity at the site of tissue injury.

Present Study:
In this patient, OKT 3 (pan T) and OKT 4 (inducer/helper T) subsets decreased moderately in the peripheral blood (Table I) but comprised the majority of the infiltrating cells in the myocardium in the acute stage of myocarditis (Fig. 2). B-cells (OK T-1) in the peripheral blood did not change markedly throughout the entire period. Thus, pan T-cells and inducer/helper T-subset may be involved in the development of myocarditis in this patient. It is possible that the transient decrease of the subsets in the peripheral blood may reflect their accumulation in the diseased heart. Markedly reduced killer cell (Leu 7) and natural killer cell (Leu 11) populations in the acute stage of myocarditis were also observed (Table I). It is evident that the distribution of peripheral and myocardial lymphocyte subsets may change in the course of the disease. In clinical settings, it may be better to consider what role(s) each T-lymphocyte subset plays in the pathogenesis of myocarditis using endomyocardial biopsy studies.

Previous Studies:
Recently, reduced suppressor T-lymphocyte activity in the peripheral blood has been noted in patients with myocarditis and dilated cardiomyopathy. It is not yet known what role(s) these T-lymphocyte subsets play in the pathogenesis of myocarditis. However, it is conceivable that the complex series of immune reactions, including T-cell mediated cytotoxicities and delayed type hypersensitivity to the causative agents, may aggravate the disease process. Some investigators who have reported cardiac immune complexes and mononuclear cell subsets in biopsy studies of patients with myocarditis have concluded that immunohistological studies are necessary for its accurate diagnosis. Establish-
ing the diagnosis of myocarditis may have therapeutic as well as prognostic implications. The predominance of OKT 4 (inducer/helper) T-cells in the diseased myocardium in the present case is not consistent with the data of Zee-Cheng et al. and Marboe et al. However, the immunological behavior of lymphocyte subsets after the onset of myocarditis may vary according to the severity of the infection, the causative agent, or the prior immune background, eg histocompatibility, of the individual.

CONCLUSION

Although we describe only one patient with myocarditis, her immunological profile, as serially examined both in the peripheral blood and in the myocardium, suggests that specific lymphocyte subsets at the site of inflammation in the acute stage of myocarditis differ from those of the corresponding peripheral lymphocytes. The disease state may result from selective homing of lymphocyte subsets to the inflammatory site or the clonal expression of the subset in response to a particular antigen in the myocardium. The transient decrease in the percentage of circulating pan T- and inducer/helper T-cells suggests a pathogenetic role in the development of myocarditis in this patient.

Acknowledgment

We thank Messrs. S. Araya, S. Iwamoto and H. Arai for their help during this work, and Dr. W. H. Abelmamn for critical reading of the manuscript.

REFERENCES


Japanese Circulation Journal Vol. 52, January 1988