Cardiac Muscle Cell Damage Through Autoimmune Mechanism
— Can cardiac proteins provoke autoimmune myocarditis? —

TOHRU IZUMI, M.D., MAKOTO KODAMA, M.D.
AND MICHIo FUJWARA, M.D.*

In this paper, the ability of human cardiac myosin to provoke autoimmune myocarditis was investigated. Myosin fractions were immunized into A.SW. mice, Lewis rats or Hartley guinea pigs. All of the immunized rats displayed overt symptoms of myocarditis and, in a few cases, died from it. The hearts of these rats were enlarged and discolored. Histologically, the muscles of the heart were characterized by remarkable cell infiltration, extensive myofiber necrosis and the appearance of polynuclear giant cells. Neither mice nor guinea pigs showed such disease profile. In this novel experimental model, the disease state was transferable by T lymphocytes. Thus, cardiac myosin was shown to provoke muscle cell damage through a T cell mediated autoimmune process.

GENERAL clinical experience suggests that humoral and/or cellular immunological disorders may be present in patients with some types of myocarditis, dilated cardiomyopathy and in post-cardiotomy patients, and those who have recently suffered a infarction. However, even now, the presence of an autoimmune myocarditis remain in doubt, because antibodies to the cardiac cellular components were known to appear only in epiphenomen of cardiac muscle damage, such as ischemia and inflammation. In some cases, autoantigens responsible for the destructive organ-specific autoimmune disease have been analyzed, both clinically and experimentally. For example, in the case of Hashimoto’s disease, thyrogblobulin is responsible for the autoimmune disease; in Insulin-dependent diabetes mellitus, islet cell cytoplasm is known to cause the disease; and in multiple sclerosis, the myelin basic protein has been identified as an autoantigen. Researchers investigating autoimmune myocarditis, however, have yet to identify a provoking protein. Some cardiac proteins have emerged as causative candidates, such as cardiac membranous proteins, myosin and actin, the ADP/ATP carrier in mitochondria, sarcomplasmic reticulum proteins, and laminin. In the present study, the possibility that myosin may induce myocarditis is investigated.

Although in the normal state this contractile protein is sequestered in the sarcoplasm, circulating anti-myosin antibodies can be clinically detected in 32% of patients with heart muscle disease probably related to immunological disorders. Myosin is known to induce myocarditis in mice.

MATERIALS AND METHODS

Cardiac myosin was isolated from normal human hearts according to the method ori-
Fig. 1. Comparison between a Lewis rat’s heart (left) immunized with cardiac myosin and a control (right) treated with normal saline.

Fig. 2. Extensive myofiber necrosis and remarkable inflammatory cell infiltrate. ×80. Hematoxylin-Eosin staining.

ginated by Murakami in 1976\textsuperscript{13} The separated cardiac proteins were composed of more than 90% heavy myosin. Experimental animals used were A.SW. mice, Lewis rats and Hartley guinea-pigs. Myosin solution was mixed with an equal amount of complete Freund’s adjuvant and was subcutaneously injected into eight week old animals at a dosage of 5 mg/kg body weight, and boosted one week later.

The histopathology of the experimental animals was investigated using conventional
methodology. The whole heart was cut to disclose a horizontal view of both the ventricles level with the papillary muscle, and was then cut to disclose a vertical view of the upper cardiac chambers. The specimens were fixed with 10% formaldehyde, embedded in paraffin, and stained with Hemotoxylin-Eosin.
**TABLE 1 CAUSATIVE CANDIDATES OF CARDIAC PROTEINS AND EXPERIMENTAL STUDIES ON AUTOIMMUNE MYOCARDITIS**

<table>
<thead>
<tr>
<th>Immunized protein and investigator</th>
<th>Date</th>
<th>Heart failure</th>
<th>Adoptive transfer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Heart extracts</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kaplan &amp; Craig</td>
<td>1963</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>Davies, Laufer et al</td>
<td>1964</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>Chaturvedi of Mehrotra</td>
<td>1967</td>
<td>(-)</td>
<td>+?</td>
</tr>
<tr>
<td>Fukuta, Kimura et al</td>
<td>1981</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>2. Membranous proteins</td>
<td></td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>Izumi, Maisch et al</td>
<td>1987</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>3. Sarcoplasmic reticulum proteins</td>
<td></td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>Acsota &amp; Santo-buch</td>
<td>1985</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>4. Myosin</td>
<td></td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>Maisch, Wittmer et al</td>
<td>1987</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>Neu, Rose et al</td>
<td>1987</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>Kodama, Izumi et al</td>
<td>1990</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Both humoral and cellular adoptive transfer experiments were performed in this study. The antimiomyosin titer was titrated by the ELISA method. When the circulating antimiomyosin titer reached a maximum, the animals were sacrificed, and sera were collected and purified as IgG fraction. The fraction was injected intravenously into syngeneic normal animals. Next, two experiments were designed for the cellular adoptive transfer. First, the effector cells were collected from the spleen or lymph nodes of the sensitized animals, i.e. those which seemed to be most seriously affected by myocarditis. The fresh cells were injected intravenously into normal syngeneic animals. Secondly, the collected spleen or lymphnode cells were incubated with Concanavaline A at 1 μg/ml for 72 h, and the collected cells were then injected into normal animals.

**RESULTS**

All the immunized Lewis rats showed clinical signs of disease: lessened their exercise and crouched in the corner of their cage on the 18th day after the first injection. Their fur become very fluffy in comparison with the saline injected control. No changes, either behavioral or histologic, were observed in the other animals (mice and guinea pigs) similarly immunized with the same protein.

Clinically, the Lewis rats documented massive pleural and pericardial effusion, ascites and congestive livers. The hearts were enlarged about two fold in weight in comparison with the controls (Fig. 1), and the surface was discolored to a yellowish-white. From this macroscopic evidence, these animals were judged to be suffering from serious myocarditis. All the rats showed the symptoms of congestive heart failure. About 5% of the rats died from low pump function. Histologically, inflammatory cells transmurally infiltrated into the cardiac wall, which is causing extensive myofiber necrosis as shown in Fig. 2. The right ventricle was more affected than the left.

The infiltrates were composed of macrophages, neutrophils and small mononuclear cells. In a corner close to the severely lesion, polynuclear giant cells appeared as shown in Fig. 3.

The IgG fraction of sera from the affected animals contained antimiomyosin antibodies, as detected by immunofluorescence (Fig. 4). The IgG fraction, in which the titer of circulating antimiomyosin was concentrated, was injected into syngeneic normal Lewis rats, but the myocarditis could not be transferred. Cellular adoptive transfer, in which fresh spleen or lymph node cells were collected from the immunized rats on the 21st day after the first injection, also failed to induce myocarditis in the syngeneic healthy rats.

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even on the histological level, although the number of injected cells which were adminis-
tered totaled one billion at maximum. On the other hand, T lymphocytes activated af-
after incubation with Concanavaline A dramatically transferred the same clinical and his-
tological myocarditis into syngeneic Lewis rats even when only 10 million cells per rat
were administered. In a few rats, the giant cells were also provoked. These adoptive
transfer experiments demonstrate that this autoimmune myocarditis is mediated by T
lymphocytes.

DISCUSSION

Experimental autoimmune myocarditis has been a great concern of cardio-immunolog-
ists for several decades (Table 1). The first experiment was undertaken by Kaplan MH
and Craig JM in 1963. They succeeded in provoking histological myocarditis in rabbits through immunization
with heterologous heart homogenates. This trial was followed by that of Davies AM and
Laufer A et al. Chatuvedi UC and Mehrotra RML stressed the inhibitory effect of neonatal thymectomy in the pathogenesis
of this condition. Fukuta S and Kimura Y et al systematized the experiment in rats. Despite
their great efforts, provocation was always limited to histological myocarditis and no clinical symptoms were observed.
However, progress made in elucidating the nature of autoimmune diseases increased the number of cardiac proteins potentially causative of myocarditis. Izumi T, Maisch B et al. tried to induce myocarditis in Balb/c and NMRI mice through immunization with cardiac membranous proteins from the human heart. The trial was only partially successful: histological cell-infiltrates occurred in only 20% of experimental NMRI mice. From the other point of view, Acsota and Santos-Buch postulated that sarcoplasmic reticulum proteins (SRA) may induce similar myocarditis. In their animal model of Chagas disease, they noticed an autoimmune cellular cytotoxicity against cardiocytes. Since 1987, attention has been focused on myosin as an antigen which may provoke autoimmune myocarditis. Maisch B and Wittner B et al. induced a histological cell-infiltrate in rabbits. Neu and Rose et al proposed a
new experimental model: an extensive myocarditis in genetically predisposed mice induced by myosin immunization. But, despite their success, myocarditis has been not adoptively transferred. In the present study, myosin proteins provoked a life-threatening autoimmune myocarditis for the first time. Our investigations have also shown that this novel experimental autoimmune myocarditis is characterized by the occurrence of giant cells in close proximity to necrotic regions, and that disease state is transferable by T lymphocytes.

From our study, myosin, out of the many proteins of which cardiac muscle is composed, is shown for the first time to be able to provoke muscle cell damage by an auto-
immune process.

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