Rat Dilated Cardiomyopathy After Autoimmune Giant Cell Myocarditis

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Abstract One of the possible causes of dilated cardiomyopathy is considered to be a sequel to myocarditis. Two mechanisms have been proposed in the process of progression of myocarditis into dilated cardiomyopathy: one is a persistent viral infection, and the other is an autoimmune myocardial injury. To clarify the possible path played by the autoimmune mechanism in the process, using an animal model, we investigated whether autoimmune myocarditis, exclusively not related to viral infection, might develop into dilated cardiomyopathy. Experimental autoimmune myocarditis was elicited in Lewis rats by immunization with cardiac myosin fraction. Rats of the control group were immunized with ovalbumin. The clinical course was observed for 4 months. Six rats from the myosin-immunized group died during the acute phase and the healing phase, and all those rats had severe myocarditis. All rats that survived until the end of the study showed enlarged and discolored hearts. Aneurysmal changes were observed in the right ventricle during thoracotomy. The ratio of heart weight to body weight of the myosin-immunized group was significantly higher than that of the control group (3.36±0.49 versus 2.69±0.06 g/kg, respectively; P<.005). The lengths of the anterior interventricular fissure and the posterior interventricular fissure of the hearts of the myosin-immunized group were significantly longer than those of the control group. The external diameter of the left ventricle of the myosin-immunized group was also significantly larger than that of the control group. Diffuse myocardial muscle loss and replacement fibrosis were the prominent histological findings of the rats of the myosin-immunized group. Inflammatory cell infiltrations disappeared from the majority of the lesions, but focal accumulations of mononuclear cells were rarely detected in the periphery of the lesions. When rats were immunized again 4 months after the initial immunization, severe myocarditis recurred in all these rats. Autoimmune giant cell myocarditis in the rat was demonstrated to develop into recurrent forms of myocarditis and to lead to dilated cardiomyopathy. This is a new model for postmyocarditis dilated cardiomyopathy not related to viral infection. (Circ Res. 1994;75:278-284.)

Key Words • myocarditis • autoimmunity • dilated cardiomyopathy • cardiac myosin • giant cell

Dilated cardiomyopathy is a set of heterogeneous diseases of left ventricular dysfunction due to unknown etiology. There are a variety of clinical courses and pathological findings.1,2 One of the possible causes of dilated cardiomyopathy is considered to be a sequel to myocarditis.3,4 Two mechanisms by which myocarditis develops into dilated cardiomyopathy have been proposed: one is a persistent viral infection, and the other is a progressive autoimmune myocardial injury.5-9 Viral myocarditis has been extensively investigated by using murine models, and it has been reported that some models will develop into postmyocarditis dilated cardiomyopathy in the chronic phase.10-12 However, definite discrimination between viral effects and autoimmune mechanisms is quite difficult in viral experimental models.13,14

Lesions associated with viral infection reveal generally lymphocytic inflammation, and granulomatous inflammation may reflect a chronic immune reaction of the host. Human myocarditis can be classified into lymphocytic myocarditis and giant cell myocarditis according to the histopathologic findings. Giant cell myocarditis was previously believed to be a rare and fatal disease of unknown etiology. Recently, it has been reported that giant cell myocarditis is more prevalent in human myocarditis than previously recognized.15 From that report, left ventricular function of patients with lymphocytic myocarditis improved during long-term follow-up. On the other hand, progressive decline of the left ventricular systolic function was observed in patients with giant cell myocarditis. That observation may imply that giant cell myocarditis is more likely than lymphocytic myocarditis to progress into dilated cardiomyopathy.

Recently, unique forms of experimental autoimmune myocarditis have been established in mice and rats by immunization with cardiac myosin.16,17 Cardiac myosin-induced rat autoimmune myocarditis is characterized by extensive myocardial necrosis, congestive heart failure, and the appearance of multinucleated giant cells and is considered to represent some part of the pathogenesis of human giant cell myocarditis.18 Myosin-induced myocarditis in the murine model and the rat model has been demonstrated to be a T cell–mediated autoimmune disease.19-20 but the precise pathogenesis of the disease is not yet resolved.21-23 These models are quite useful in the analysis of the mechanisms of autoimmune myocardial injury.24-26

To clarify the role of autoimmunity in the process of progression of myocarditis into dilated cardiomyopathy, clinicopathological findings of rat experimental autoimmune myocarditis, which was exclusively not related to viral infection, was investigated in the chronic phase.
Materials and Methods

Animals

Lewis rats were purchased from Charles River Japan Inc, Atsugi, Kanagawa, Japan, and maintained under the specific-pathogen-free condition at The Facilities for Comparative Medicine & Animal Experimentation, Niigata University School of Medicine.

Antigen

Purified cardiac myosin was used as the antigen. Cardiac myosin was prepared from the ventricular muscle of human hearts. The purification procedure has been previously described.17

Immunization

The antigen was dissolved in a solution of 0.3 mol/L KCl and 0.2 mol/L phosphate-buffered saline at a concentration of 10.0 mg/mL. Eight-week-old male rats were immunized with 1.0 mg cardiac myosin in an equal volume of Freund’s complete adjuvant containing 6.0 mg/mL Mycobacterium tuberculosis (group A, n=17). Rats of the control group were immunized with 1.0 mg ovalbumin (OVA) in the same manner (group B, n=12). Rats were immunized again after an interval of 7 days. The morbidity of experimental autoimmune myocarditis was 100% in rats immunized when using this protocol.20

Experimental Period and Sampling

The clinical course was observed for 4 months, and then the rats were killed under ether anesthesia. Heart, lung, liver, and spleen weights were measured immediately after death. The ratios of organ weight (in grams) to body weight (in kilograms) were calculated.

Measurement of Heart Size

Heart size of each rat was evaluated by three scales measured after formalin fixation. The first was the length from the apex to the aortic root through the anterior interventricular fissure (anterior). The second was the length from the apex to the atioventricular fissure through the posterior interventricular fissure (posterior). The third was the external short-axis diameter of the left ventricle (short axis).

Histopathology

Hearts were removed immediately after killing and fixed in 10% formalin. After embedding in paraffin, several transverse sections were cut from the ventricle and stained with hematoxylin-eosin and Azan-Mallory methods. Histopathology was investigated at the end of the study in the rats in groups A and B. Pathological changes of cardiac lesions during the disease course were also investigated at days 11, 14, 17, 21, 28, 42, and 84 in rats that were immunized with cardiac myosin. The control group consisted of the rats immunized with OVA.

Immunohistochemistry

Bound IgG in the heart was investigated by direct immunohistochemical staining using peroxidase-conjugated goat anti-rat IgG (Cappel). Sections taken at days 11, 21, 42, and 84 were cut in a cryostat from rat hearts of both the myosin-immunized group and the OVA-immunized group. The staining methods were previously described.26

Anti–Cardiac Myosin Antibodies

Anti–cardiac myosin antibodies in sera from rats of groups A and B were measured by using an enzyme-linked immunosorbent assay as previously described.17 Circulating anti–cardiac myosin antibodies were also measured in rats that were immunized with cardiac myosin and killed on day 21 (n=5).

Results

Clinical Course

Rats of the myosin-immunized group became ill and immobile at the third week, and then their activity gradually recovered beginning at the fifth week. Six rats (35%) of the myosin-immunized group died from day 19 to day 39 (Fig 1). Four rats died during the acute phase, and two died during the healing phase. All hearts from these rats showed extensive myocardial necrosis.

Macroscopic Findings

At thoracotomy, some rats of the myosin-immunized group showed aneurysmal changes in the right ventricular outflow tract. There was no pericardial effusion in either group. The hearts of the rats of the myosin-immunized group were enlarged. Discolored (whitish) areas were diffusely scattered over the entire surface of their hearts. Both ventricles were dilated in the myosin-immunized group (Fig 2). The left ventricular wall of the myosin-immunized rats was thinner than that of the control rats. The macroscopic appearance of the hearts of the rats in the control group was normal.

Organ Weights

The ratio of heart weight to body weight of the myosin-immunized group was significantly higher than...
that of the control group (3.36±0.49 versus 2.69±0.06 g/kg, respectively; *P*<.005) (Fig 3). There were no differences between the myosin-immunized and the control groups concerning the ratios of lung weight to body weight (3.56±0.24 versus 3.63±0.21 g/kg, respectively), liver weight to body weight (31.4±2.2 versus 30.8±1.4 g/kg, respectively), or spleen weight to body weight (2.34±0.78 versus 2.07±0.04 g/kg, respectively).

**Heart Size**

Macroscopically, the hearts of the rats of the myosin-immunized group were markedly enlarged. The length from apex to aortic root (anterior) of the myosin-immunized group was significantly longer than that of the control group (17.9±0.72 versus 16.2±0.63 mm, respectively; *P*<.001) (Fig 4). The length of posterior interventricular fissure of the myosin-immunized group was also significantly longer than that of the control group (12.8±0.54 versus 11.9±0.52 mm, respectively; *P*<.001). Further, the short-axis external left ventricular diameter was significantly larger than that of the control group (13.7±1.05 versus 11.9±0.72 mm, respectively; *P*<.001).

**Histopathology**

Various levels of transverse sections of the ventricles were examined by use of light microscopy. A prominent histological finding of the myosin-immunized group was fibrosis, especially replacement fibrosis. Fibrosis spread diffusely into the entire myocardium of the hearts (Fig 5A). The most frequently involved area was the subepicardial layer. Occasionally, there were segmental and transmural lesions or subendocardial lesions. The distributions of fibrosis did not depend on the coronary perfusion territories.

Dense collagen fibers consisted of replacement fibrosis. Interstitial fibrosis spread widely into the surrounding myocardium. Hypertrophic or atrophic myocardial fibers were observed in and around the lesions. Some cardiomyocytes showed degeneration. Arrangement of
the myocardial fibers in the lesions was disturbed. However, myocardial fibers existing far distant from the lesions showed normal appearance.

Focal accumulations of mononuclear cells were rarely detected in the periphery of the lesions (Fig 5B). However, the proportion of the area of mononuclear cell accumulation was very small compared with the area of fibrosis.

Pathological Findings up to 4 Months

Rats killed on day 11 showed no abnormality in their hearts. Severe myocarditis characterized by the appearance of multinucleated giant cells was observed in all rats killed on day 14. Fulminant myocarditis continued until day 28. Histological findings of the rats killed on days 42 and 84 revealed healed myocarditis, namely, the disappearance of infiltrating mononuclear cells and prominent fibrous tissue replacing the area of myocardial necrosis.

Anti-Cardiac Myosin Antibodies and Bound IgG

Anti-cardiac myosin antibodies were measured in the sera of three groups of rats: the first group was composed of myosin-immunized rats killed on day 21, the second was group A, and the third was group B. The optical density values of the three groups were 0.721±0.124, 0.552±0.142, and 0.220±0.0362, respectively. The value of group A, the chronic-phase group, was significantly lower than that of the acute-phase group (P<.05) but was still significantly higher than that of the control group (P<.001).

Bound IgG on the surface of myocardial fibers was investigated in the rats killed on days 11, 21, 42, and 84. Although degenerated or necrotic areas were stained with anti-rat IgG antibody, cardiomyocytes in the intact areas were not stained throughout the course of the disease. No difference was observed in the staining between intact areas of diseased hearts and control hearts.

Recurrence

All rats that were immunized with cardiac myosin for a second time after a 4-month interval showed severe active myocarditis (Table). Pericardial effusion was observed in two rats after death. Myocardial necrosis and
mononuclear cell infiltrations were observed with widespread fibrosis (Fig 6). Three of four rats immunized with cardiac myosin after a 2-month interval revealed severe active myocarditis. Severe recurrence of experimental autoimmune myocarditis was also observed in a rat from the group immunized after a 1-month interval.

**Discussion**

Cardiac myosin–induced autoimmune myocarditis, which was exclusively not related to viral infection, was demonstrated to progress into the clinicopathological state similar to dilated cardiomyopathy in the chronic phase. Pathological findings in this model of postmyocarditis dilated cardiomyopathy, such as enlargement of the heart, dilatation of both ventricles, diffuse and extensive myocardial fibrosis, and hypertrophic and atrophic changes of myocardial fibers, resembled human dilated cardiomyopathy.

Human dilated cardiomyopathy is thought to be due to a variety of causes. Therefore, clinical courses and pathological findings in dilated cardiomyopathy are not uniform. Several animal models of dilated cardiomyopathy have previously been established, and these models have both similar and different features compared with human dilated cardiomyopathy. Different findings concerning the clinical course were also noticed in this model and in human dilated cardiomyopathy. First, dilated cardiomyopathy in the rat model occurs after the apparent acute phase, which is usually absent in human dilated cardiomyopathy. Second, the rat model shows a monophasic and nonprogressive clinical course. Most human dilated cardiomyopathy is considered to have a progressive nature of myocardial damage, even if significant exceptions exist. Rats with autoimmune myocarditis died because of congestive heart failure in the acute phase and in the healing phase; no rats died during the chronic phase in the present study. Therefore, this model of dilated cardiomyopathy seems to represent the pathogenesis of some but not all human dilated cardiomyopathy.

Rat dilated cardiomyopathy shows several pathological features that have not been described in other animal models of dilated cardiomyopathy. First, gross pathology revealed aneurysmal changes of the right ventricle. Idiopathic ventricular aneurysm has been occasionally observed in humans and, when preceding myocarditis, seems to be one of its causes. Aneurysmal change represents transmural myocardial damage and inadequate healing process. Second, myocardial lesions of this rat model had no calcification, which is frequently observed in murine dilated cardiomyopathy after viral myocarditis. Human dilated cardiomyopathy also rarely reveals calcifications in the lesions.

Rarely, small accumulations of mononuclear cells were observed in this model of postmyocarditis dilated cardiomyopathy. Clinically unsuspected inflammatory cell accumulations have been occasionally discovered by endomyocardial biopsy in patients with unexplained congestive heart failure and have recently been diagnosed as idiopathic dilated cardiomyopathy. It is

<table>
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<tr>
<th>Group</th>
<th>First Ag</th>
<th>Interval</th>
<th>Second Ag</th>
<th>n</th>
<th>Heart/Body Weight</th>
<th>PE</th>
<th>Macro</th>
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<td>M1</td>
<td>M</td>
<td>1 mo</td>
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<td>5</td>
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<td>2 mo</td>
<td>M</td>
<td>4</td>
<td>4.56±0.76†</td>
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<td>4</td>
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<tr>
<td>OVA4</td>
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<td>4</td>
<td>2.74±0.06</td>
<td>0/4</td>
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Ag indicates antigen; n, number of rats; PE, pericardial effusion; Macro, macroscopic score; Micro, microscopic score estimated by the area of mononuclear cell infiltration; M, myosin; and OVA, ovalbumin.

The scoring systems were previously described.18

*P<.01, †P<.005, and ‡P<.02 vs the OVA4 group.

![Fig 6. Photomicrograph shows histopathology of the heart of a rat reimmunized with cardiac myosin after a 4-month interval. Widespread fibrosis existed in the heart, and active myocarditis recurred. The histological findings of recurrent myocarditis were characterized by the coexistence of fibrosis and active inflammation (Azan-Mallory staining; bar=100 μm).](image-url)
still controversial whether those cell infiltrates indicate etiologic relevance of myocarditis to dilated cardiomyopathy or the nonspecific pathological findings of myocardial damage of dilated cardiomyopathy. This observation implies that focal accumulations of mononuclear cells were preceded by myocarditis.

Both viral myocarditis and autoimmune myocarditis show a self-limiting nature. What is the predictor of recovery or progression? Myocardial muscle damage preferentially occurs during the acute phase. Because significant left ventricular fibrotic changes have already been produced in postmyocarditic hearts, some stressful conditions, such as hypertension, tachycardia, alcohol, and nonspecific inflammatory chemical mediators, may easily create congestive heart failure or progressive left ventricular dysfunction after myocarditis.36,37 If this occurs, myocarditis alone may not progress into typical dilated cardiomyopathy, but various worsening factors may create dilated cardiomyopathy in patients with postmyocarditis nonclinical left ventricular dysfunction. Another possibility is persistent or recurrent myocarditis. Viral myocarditis usually reveals a short clinical course, and recurrence of myocarditis due to the same viruses has never been observed. Secondary infection by another virus can elicit secondary myocarditis,38,39 but the possibility and frequency of these conditions are unclear in human cases. The present study has demonstrated that autoimmune myocarditis is easily able to recur by the same antigen challenge. Therefore, when an immune regulatory system against auto-reactive T cells is disturbed by exposure to foreign mimic antigens or sequestered self antigens, patients with autoimmune myocarditis may develop recurrent myocarditis that leads to dilated cardiomyopathy. Various cytokines have also been demonstrated to play an important role in the disturbance of the immune regulatory system and the initiation of cardiac myosin–induced autoimmune myocarditis.40-42

The unique finding of the present study is that the initial myocarditis leading to dilated cardiomyopathy is a giant cell myocarditis and that this rat giant cell myocarditis is an autoimmune disease against cardiac myosin. The presence of circulating anti-heart antibodies in the sera of patients with dilated cardiomyopathy has been repeatedly reported, and evidence supports the hypothesis of autoimmune mechanisms in the pathogenesis of dilated cardiomyopathy.43-46 A recent study has revealed that major autoantigens in dilated cardiomyopathy are cardiac myosin heavy chain isoforms.47 Therefore, the fact that cardiac myosin–induced autoimmune giant cell myocarditis leads to dilated cardiomyopathy may represent the situation for the human form of this disease.

Several possible mechanisms have been proposed in the process of the progression from myocarditis to dilated cardiomyopathy. The first is a persistent viral infection and metabolic disturbance of cardiomyocytes due to the existence or replication of viruses. The second is a persistent viral infection and antiviral immune response that is solely beneficial to the host but harmful to the organs lacking regenerative activity. The third is host-immune reactions against the viral antigen or self antigen mimicking the viral epitope after the clearance of viruses. The present study demonstrated for the first time that autoimmune myocarditis, exclusively not related to viral infection, might develop into dilated cardiomyopathy in the chronic phase. Recently, great attention has been focused on persistent viral infection,38,44 but we suggest that autoimmune mechanisms should be considered in some patients with dilated cardiomyopathy. This new animal model may serve as a useful tool in the investigation of the pathogenesis of chronic inflammatory myocardial damage leading to dilated cardiomyopathy.

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