

# Experimental Autoimmune Myocarditis

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Received December 12, 1990

**Summary.** Recently, we have established a novel experimental model for autoimmune myocarditis. Acute serious myocarditis can be induced in Lewis rats by immunization with cardiac myosin. This experimental myocarditis is characterized by congestive heart failure. As is the case of acute myocarditis in humans, massive pericardial effusion, swelling and discoloration of the cardiac wall can be observed in this model. The lesions are composed of various inflammatory components, such as neutrophils, lymphocytes, macrophages, fragments of degenerated myofibers and interstitial edema. A considerable number of multinucleated giant cells are also observed in the lesions. This myocarditis is transferable in syngeneic naive rats by injection with concanavalin A-activated spleen cells obtained from previously immunized rats. On the other hand, the immunoglobulin fraction from rats with severe myocarditis is unable to transfer the myocarditis. Immunohistochemically, the cell infiltrates are mainly composed of macrophages and CD4+ T cells. Other populations of T cells are scarce and B cells are absent from this myocarditis. Microscopically, multinucleated giant cells were of two types, these being macrophage-like and myocyte-like in appearance. Immunohistochemically, both of them were stained with only OX42, a macrophage marker, and were not stained with an anti-desmin antibody nor any lymphocyte markers. Consequently, we deduced that this experimental myocarditis is mediated by T cells. This model is expected to provide important information for the pathogenesis of human myocarditis.

## INTRODUCTION

Immune reactions of a host have been considered to be involved in the pathogenesis of several heart diseases, such as rheumatic carditis,<sup>1)</sup> chronic Chagas' disease,<sup>2)</sup> Dressler syndrome and postpericardiotomy syndrome.<sup>3,4)</sup> Recently, the involvement of autoimmune reactions has come to be considered as

a cause of myocarditis and dilated cardiomyopathy.<sup>5-8)</sup> Thus, analyses of immunological mechanisms in cardiac diseases are important, as is the establishment of adequate animal models corresponding to each disease.

Various infectious organisms and toxic exposures are able to produce inflammatory lesions in human hearts.<sup>9)</sup> The most frequent etiology of human myocarditis is proposed to be viral infections, especially Coxsackie viruses.<sup>10)</sup> However, it is difficult to make an accurate diagnosis of myocarditis by clinical examination. Pathogens such as viruses are rarely detected in samples from patients, even during the active phase. It is uncertain why the pathogen can rarely be detected in human myocarditis. One possibility is suggested by experimental evidence that autoimmunity triggered by viral infection acts in myocarditis.<sup>11)</sup>

Hypersensitivity myocarditis and giant cell myocarditis are entities distinct from infectious or toxic myocarditis. The former type of myocarditis is considered to contain a delayed-type of hypersensitivity process against various agents, such as penicillin and methyldopa.<sup>12)</sup> Concerning giant cell myocarditis, circulating anti-heart antibodies are occasionally remarkable.<sup>13)</sup> Clinically the autoimmune mechanism is proposed as a pathogenesis of these myocarditis, but, until now, no adequate animal models for these diseases have been presented.

Dilated cardiomyopathy (DCM) is considered a set of heterogeneous diseases. One possible cause of DCM is proposed to be the sequela of myocarditis.<sup>14)</sup> The mechanisms by which myocarditis develops to DCM are not fully elucidated. Many experimental studies using murine viral myocarditis have been performed to clarify this process. From those studies, the generation of auto-reactive cytotoxic T-cells has been demonstrated *in vitro*.<sup>15)</sup> To clarify the pathogenesis of DCM, experimental autoimmune myocar-

ditis will become useful.

Our chief interests are as follows. Firstly, are there actually cases of autoimmune myocarditis?<sup>16)</sup> Secondly, is autoimmunity responsible for the progression to dilated cardiomyopathy from myocarditis? To answer these questions, we have made efforts to establish experimental autoimmune myocarditis.

### Previously reported experimental autoimmune myocarditis

During the last three decades, many experimental autoimmune myocarditis have been reported (Table 1).<sup>17-32)</sup> From 1958, M. H. Kaplan et al. investigated autoimmune myocardial disease by the sensitization of rabbits with heterologous heart homogenate. They detected the production of circulating auto-reactive antibodies and focal mononuclear cell infiltration in the myocardium. Thereafter, various antigens such as heart homogenate, extract, sarcoplasmic reticulum proteins, membranous proteins and cardiac

myosin, were used to induce myocarditis in various animals. However, none of these models was fully satisfactory from clinical point of view, because they showed rather mild lesions of only microscopic change. In this way, they differed from human myocarditis, which is frequently evidenced by symptoms of congestive heart failure and occasionally by a fatal clinical course. Another problem of these models has been the incomplete induction of myocarditis, even when all animals were immunized with the same procedures. For the investigation of its clinical course or for the therapy of the disease, it is desirable that a homogeneous disease is induced in all of the sensitized animals without exception.

### Susceptibility to autoimmune myocarditis

In the induction of organ-specific autoimmune diseases, the relationship between the antigens and the host animal is of considerable importance. The ideal combination for myocarditis remains uncertain, how-

**Table 1.** Experimental autoimmune myocarditis

Reporter	animal	antigen	results (myocarditis) <sup>1)</sup>
1958 Kaplan	rabbit	het. HH	0%, autoantibodies
1963 Kaplan	rabbit	het. HH	10%, histologic
1964 Davies	rabbit	het. and	32%, histologic
	rat (albino)	hom. HH	
	guinea pig hamster		
1966 Gazenfeld	rat (albino)	hom. HH + ISP	46%, histologic, severe
1967 Chaturvedi	rat (albino)	het. EH	ND histologic, transfer with spleen
1968 Halbert	rabbit	het. and hom. HH	0%, autoantibodies, high mortality
1970 Friedman	rat (Lewis)	het. and hom. HH	36%, histologic
1980 Fukuta	rat (Wister)	hom. EH	86%, histologic
1981 Fukuta	rat (Wister)	het. EH	100%, histologic
1983 Wittner	rabbit	het. CM	ND histologic
1984 Cossio	mouse (Swiss)	hom. HH	89%, histologic, sudden death
1984 Meckert	mouse (Swiss)	SFCF	38%, histologic
1985 Acosta	mouse (BALB/c)	SRA	78%, histologic
1985 Hosenpud	guinea pig	het. HH	78%, histologic, increased LV weight
1987 Izumi	mouse (NMRI)	het. and hom. MP	20%, histologic
1987 Neu	mouse (A/J)	hom. CM	85%, severe, swelling of the hearts
	(A. SW)		
	(A. CA)		
1990 Kodama	rat (Lewis)	het. and hom. CM	100%, severe, giant cell, heart failure

Abbreviations, het.: heterologous, hom.: homologous, HH: heart homogenate, EH: extract of heart, ISP: isoproterenol, CM: cardiac myosin, SFCF: subcellular fraction from *Crithidia fasciculata*, SRA: sarcoplasmic reticulum antigen, MP: cardiac membranous protein, ND: not described.

1) The frequency of myocarditis in treated animals is given in percentages.

ever. We have investigated susceptibility to autoimmune myocarditis using various animals, i.e., mice (A.SW), rats (Lewis, BN and PVG), and guinea pigs (Hartley). Heart extracts from humans, rats and mice were prepared as antigens. Purified cardiac myosin was also tested. Cardiac myosin was prepared from the ventricular muscles of human and rat hearts. About 90% of this preparation was demonstrated to be cardiac myosin, though several other substances were actually present. Animals were immunized with the antigen in complete Freund's adjuvant on days 0 and 7.

As a result, the only effective combination for the induction of myocarditis was the immunization of Lewis rats with cardiac myosin from either rats or human (Table 2).<sup>33)</sup> Both cardiac myosins of the human and rat induced a similar type of myocarditis by macroscopic and histologic observation. Most importantly, every rat immunized showed severe myocarditis without exception. No myocarditis was induced by sensitization with heart extracts, even in Lewis rats.

**Table 2.** Susceptibility of animals to autoimmune myocarditis<sup>1)</sup>

Animal	heart homogenate		cardiac myosin	
	het.	hom.	het.	hom.
mouse (A. SW)		0/4	0/13	
(A. BY)		0/4		
rat (Lewis)	0/10	0/5	40/40	3/3
BN)			0/8	
PVG)			0/8	
guinea pig		0/10		

Abbreviations, het.: heterologous, hom.: homologous.  
1) Animals were immunized with the proper antigen shown in the table. Data indicate animals showing myocarditis/total number of treated animals.

Hartley guinea pigs have been known to be highly susceptible to experimental allergic encephalomyelitis (EAE) but proved resistant to myocarditis. It has also been demonstrated that severe myocarditis can be induced in A.SW mice by immunization with murine cardiac myosin.<sup>22)</sup> However, myocarditis was not induced by either human nor rat cardiac myosin in these animals in our experiments. The exact immune mechanisms which determine the susceptibility to autoimmune myocarditis remain unknown. In cases of EAE or experimental autoimmune thyroiditis, susceptibility is related to the major histocompatible complex. Similar genetic restrictions

of susceptibility may exist in autoimmune myocarditis.<sup>34)</sup>

Because the antigen in our study was a crude cardiac myosin, the actual myocarditogenic substance for autoimmune myocarditis was not determined. As already shown in EAE or other organ-specific autoimmune diseases, determination of the amino acid sequence of an antigen is invaluable for clinical application.<sup>35,36)</sup> For precise analyses of autoimmune myocarditis, detection of actual myocarditogenic peptides is required.

### Pathology of a novel experimental autoimmune myocarditis

The similarity in histopathology between human cases and experimentally induced lesions is essential for clinical references of experimental models. Pathological findings were examined in rats immunized with cardiac myosin.

Lewis rats immunized with human cardiac myosin developed severe myocarditis on day 21 after the first immunization. This myocarditis was characterized by macroscopic changes in the hearts, such as pericardial effusion, marked enlargement of the hearts and gray discoloration of the cardiac muscle (Fig. 1). Such macroscopic changes are frequently observed in autopsied human hearts with acute myocarditis. Some of the rats with myocarditis died during the observation period, probably from congestive heart failure.

Inflammatory lesions were composed of mononuclear cells, polymorphonuclear cells and fragments of necrotic myofibers. Extensive myocardial necrosis and interstitial edema were also observed (Fig. 2). Most interestingly, a considerable number of multinucleated giant cells were detected in the lesions (Fig. 3).<sup>27)</sup>

To our knowledge, there have been no reports of experimental giant cell myocarditis. This experimental myocarditis may become a unique tool to examine the pathogenesis of human giant cell myocarditis.

### Clinical course and production of anti-myosin antibodies in experimental autoimmune myocarditis

In human giant cell myocarditis, definite diagnosis can barely be made at autopsy. Patients with this disease die within a few days to several months after the onset of symptoms. The precise clinical course remains unclear. We weekly investigated the histopathology of the experimental myocarditis. The production of circulating anti-myosin antibodies was



**Fig. 1.** On Day 21 after the first immunization, massive pericardial effusion is observed in immunized rats. The heart is markedly enlarged. The color of diseased myocardium has changed to gray.

also measured by an enzyme-linked immunosorbent assay.

This experimental myocarditis developed from 16 to 18 days after the first immunization. Immediately after the onset, the rats showed a fulminant phase until the fifth week; some of them died during this period. By the sixth week inflammation become smoldering and the lesions were replaced by fibrosis. The titer of anti-myosin antibodies was raised prior to the development of myocarditis, and maintained a high level until the sixth week (Fig. 4).

The surviving rats recovered spontaneously from myocarditis in this model. The mechanism of recovery from myocarditis may provide information concerning possible therapy for human myocarditis.

#### **T cell-mediated autoimmune disease**

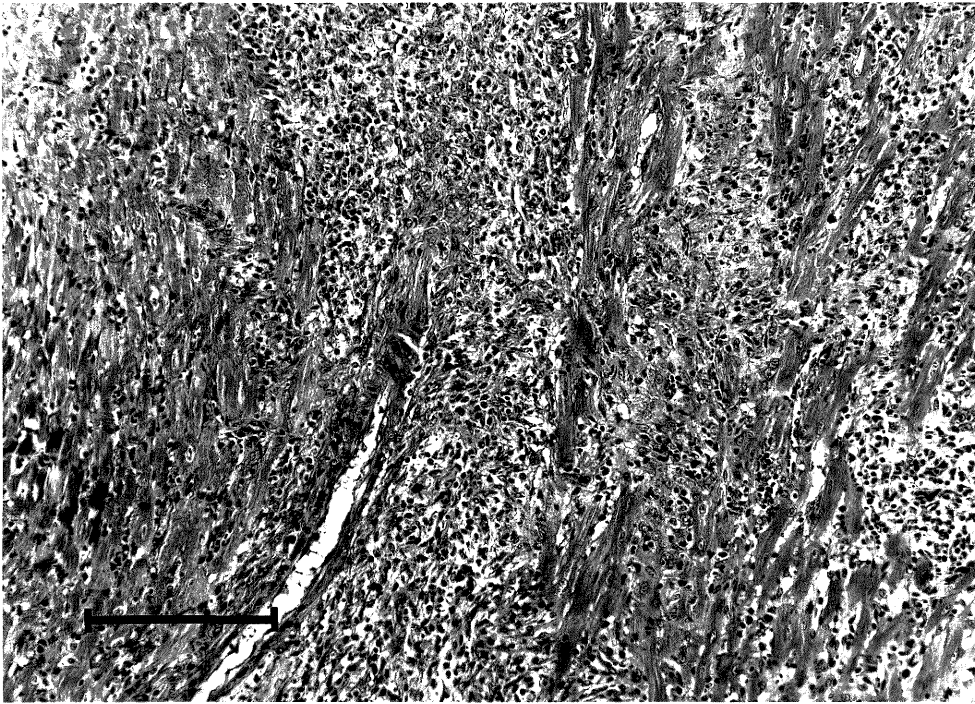
Some of experimentally induced autoimmune diseases are known to be transferable into naive syngeneic animals with antibodies or effector T cells. As far as experimental autoimmune myocarditis is concerned, adoptive transfer has not previously been

reported either with autoantibodies or lymphocytes. Therefore, the actual effector component has not been determined. This might be due to incomplete priming upon active immunization. We demonstrated that severe myocarditis was transferable into syngeneic rats by injection of concanavalin A-activated spleen cells from primed rats.<sup>38)</sup>

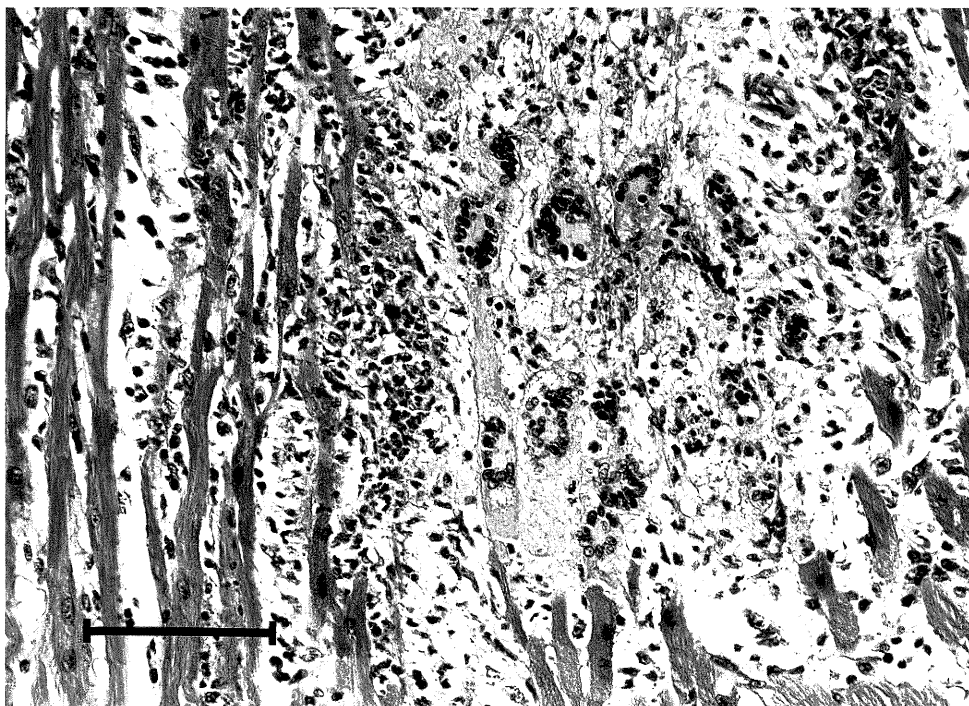
An immunoglobulin fraction was prepared from rats with severe myocarditis by ammonium sulfate at 40% saturation fractionation. The fraction was injected intraperitoneally in naive Lewis rats. Lectin-activated lymphocytes were also prepared from the spleen cells of primed rats by incubation with 1  $\mu\text{g}/\text{ml}$  of concanavalin A. The activated lymphocytes, namely T cells, were injected intravenously into the syngeneic rats.

As the result, only the rats injected with activated T cells demonstrated severe myocarditis (Fig. 5). Multinucleated giant cells were observed in transferred myocarditis. The rats injected with the immunoglobulin fraction or freshly prepared lymphocytes did not show myocarditis at all.

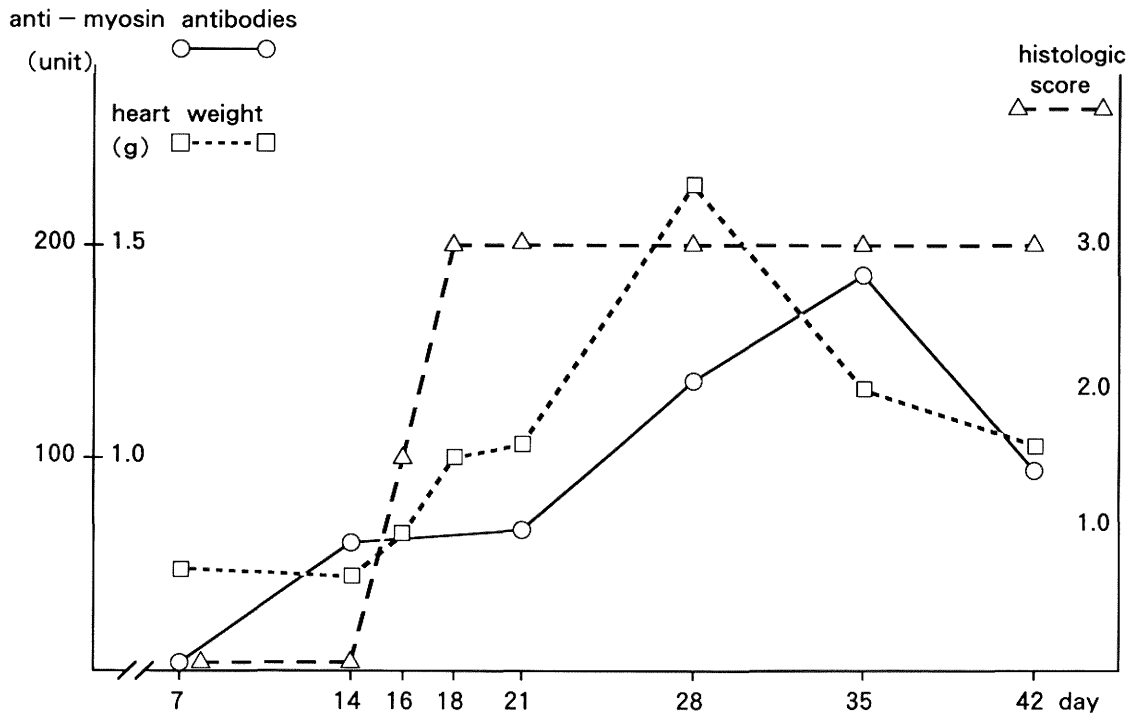
EAE has been demonstrated to be a CD4+ T



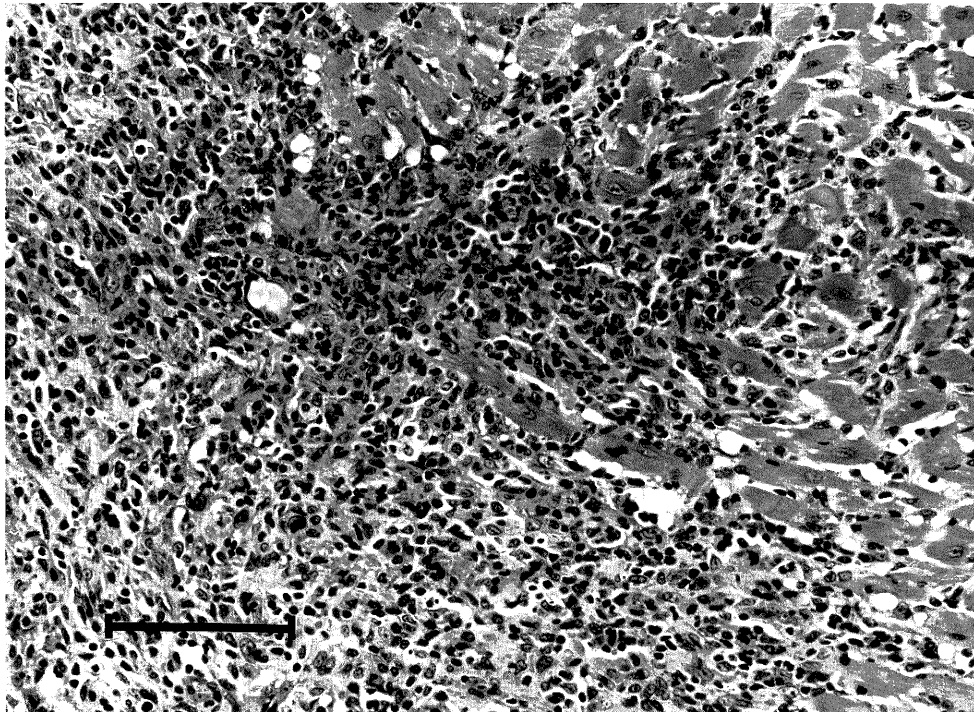
**Fig. 2.** Inflammatory cell infiltration and extensive myocardial necrosis are observed in the macroscopically discolored area. The bar indicates 200  $\mu\text{m}$ .



**Fig. 3.** Multinucleated giant cells frequently appear in the lesions of this myocarditis. The bar indicates 100  $\mu\text{m}$ .



**Fig. 4.** Clinical course of experimental autoimmune myocarditis. Histologic score was defined as follows: normal—0, a few small and focal lesions—1, moderately large lesions—2, diffuse or broad lesions—3.



**Fig. 5.** On Day 11 after injection of lymphocytes, severe myocarditis can be observed. As shown in actively induced myocarditis, inflammatory cell infiltration and extensive myocardial necrosis are present. The bar indicates 100  $\mu$ m.

cell-mediated autoimmune disease, and encephalitogenic clones have been obtained.<sup>39,40)</sup> Other organ-specific autoimmune diseases, such as experimental autoimmune neuritis, arthritis, thyroiditis, uveoretinitis and orchitis, are regarded as an entity similar to EAE, and these diseases are also transferable with CD4+ T cells.<sup>41-45)</sup> Experimental autoimmune myocarditis is induced by the same procedures as those diseases and is transferable with lectin-activated spleen cells. Therefore, this myocarditis may also be mediated by CD4+ T cells. Clonal analyses of effector lymphocytes will be important for elucidating autoimmune myocarditis.

### Immunohistochemical analysis

To clarify the characteristics of autoimmune myocarditis, immunohistochemical analysis was carried out using monoclonal antibodies specific for rat lymphocytes, macrophages and the MHC molecules.<sup>46)</sup> Frozen sections of the hearts were incubated with W3/25 (helper T cells), OX8 (cytotoxic / suppressor T cells), OX19 (pan T cells), OX33 (B cells), OX42 (macrophages), OX18 (MHC class I) and OX6 (MHC class II). Positive staining was detected by avidin-biotin complex methods.

About 70% of the infiltrated cells in the lesions were composed of OX42-positive cells, namely macrophages. About 20% of the infiltrates were W3/25-stained T cells. OX8-positive T cells comprised a small population in the lesions, and B cells were rare. Therefore, it seems that macrophages and CD4+ T cells play important roles in the development of autoimmune myocarditis.

Normally, myofibers do not express MHC class II antigens on the membrane. MHC Class I antigens are also not detectable on myocytes by conventional immuno-staining methods. A considerable number of class II-positive dendritic cells were observed in the interstitium of the heart of normal rats. At the onset of myocarditis, interstitial dendritic cells markedly increased in number. Because this type of cell plays the role of antigen presenting cells, autoimmune myocarditis may be regulated by these cells.

### Characterization of giant cells

The origin of multinucleated giant cells in myocarditis is an interesting but controversial issue. Previously, many investigators proposed that giant cells were derived from degenerated myocardial fibers.<sup>47-50)</sup> Morphological evidence supported this myogenic theory: for example, the large and rod-

shaped morphology of the cytoplasm lying parallel to the surrounding muscle fibers or the presence of myocardial cell components in the cytoplasm of giant cells. Recently, there have been a few reports indicating the presence of a macrophage-specific antigen on the membrane of giant cells.<sup>51,52)</sup> Therefore, we examined the characteristics of the giant cells of this myocarditis.

Although both types of giant cells, those being myocyte-like and macrophage-like in appearance, were observed in the lesions of this myocarditis, they were only stained with OX42, a macrophage marker, and were not stained with the anti-desmin antibody, a marker for muscle cells, or any lymphocyte markers.<sup>53)</sup> Therefore, we can deduce that the multinucleated giant cells which appeared in this model were derived from macrophages.

As yet, it can not be determined what process provoke the formation of giant cells in myocarditis, our model may provide information concerning the problem.

### Clinical implications

Human myocarditis has not yet been classified etiologically. We have here demonstrated evidence that severe myocarditis can be induced by autoimmune processes. In the case of giant cell myocarditis and idiopathic myocarditis with circulating anti-heart antibodies, autoimmune involvement might be considered.

Recently, immunosuppressive therapy for active myocarditis has been applied to clinical medicine.<sup>54,55)</sup> However, the benefit of immunosuppressive therapy is now considered controversial. One of the reasons seems to be related to the selection of patients. As active myocarditis is a clinical entity of myocarditis, it may be of various etiologies. Only those patients with autoimmune involvement must be selected for immunosuppressive therapy. Clarifying the characteristics of autoimmune myocarditis is helpful for making this selection. Furthermore, the selection and combination of immunosuppressants are important for practical use. Experimental studies can answer these problems.<sup>56)</sup>

Progression from myocarditis to dilated cardiomyopathy is clinically an interesting issue. Autoimmune myocardial injuries are proposed as the chief mechanism for this progression. This experimental myocarditis may provide a significant clue concerning this problem.

## CONCLUSION

We have established a novel experimental autoimmune myocarditis characterized by the appearance of multinucleated giant cells. Clinical and histologic characteristics of this model were discussed in their relevance to human myocarditis. The mechanisms of autoimmune myocardial injuries can be investigated using this animal myocarditis. This experimental autoimmune myocarditis may become a useful model for the elucidation of human myocarditis.

**Acknowledgments.** We thank Dr. Yoh Matsumoto and Prof. Michio Fujiwara for their valuable advice and cooperation in this study. We also thank Prof. Akira Shibata for his support throughout this study.

This study was supported in part by a grant in aid from The Tsukada Medical Science Foundation and a grant for scientific research (No. 01570476) from The Ministry of Education, Science and Culture of Japan.

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