Characteristics of Giant Cells and Factors Related to the Formation of Giant Cells in Myocarditis

Makoto Kodama, Yoh Matsumoto, Michio Fujiwara, Shaosong Zhang, Haruo Hanawa, Eiichi Itoh, Takashi Tsuda, Tohru Izumi, and Akira Shibata

Giant cell myocarditis is a serious and frequently fatal inflammatory heart disease of which the etiology remains unknown. In the present study, we investigated the origin of multinucleated giant cells in myocarditis with the use of an experimental model. We also examined the factors relating to the formation of giant cells in myocarditis. Severe myocarditis characterized by the appearance of multinucleated giant cells was induced in Lewis rats by immunization with cardiac myosin in complete Freund’s adjuvant. Two types of giant cells, foreign body giant cell–like and myocyte-like, were observed in this myocarditis. Immunohistochemical studies revealed that both types of multinucleated giant cells were stained with OX42 and ED1 (macrophage markers) and were not stained with anti-desmin antibody and HHF35 (markers for muscle fibers). Therefore, it is likely that multinucleated giant cells in this myocarditis are derived from macrophages. During the course of the disease, the appearance of multinucleated giant cells was restricted to a period corresponding with the fulminant phase of inflammation. When the severity of the disease was modulated by immunization with various doses of the antigen, multinucleated giant cells appeared only in severe myocarditis after inoculation of a large dose of the antigen. Administration of immunoadjuvants also affected the formation of giant cells. Most of the rats injected with cardiac myosin in complete Freund’s adjuvant developed giant cell myocarditis. When rats were immunized with the antigen in incomplete Freund’s adjuvant followed by a Bordetella pertussis injection, all the rats developed myocarditis without giant cells. Not only myocarditis but also the formation of giant cells was transferable into syngeneic rats with lectin-activated spleen cells taken from rats previously immunized with cardiac myosin in complete Freund’s adjuvant. Based on these findings, strongly activated macrophages or T lymphocytes seem to be necessary for the generation of giant cell myocarditis. (Circulation Research 1991;69:1042–1050)

Giant cell myocarditis is a rare but severe inflammatory heart disease and frequently takes a fatal course.1–4 Diagnostic criteria of the disease are somewhat obscure, and the etiology of the disease has remained unclear.5–10 It is also uncertain whether giant cell myocarditis is an actual homogeneous disease entity. There is a possibility that the appearance of giant cells may be one of pathological characteristics of acute myocarditis under various etiologies.

The origin of multinucleated giant cells in myocarditis is an interesting and controversial topic. Light and electron microscopic studies support the possibility that giant cells are of myogenic origin.11,12 Recently, one group reported that multinucleated giant cells were immunohistochemically positive for anti-macrophage antibody.13 However, there have been very few reports on this issue because the disease is rarely encountered. Moreover, investigation of the pathogenesis of this disease has been very difficult.

Recently, we have produced a novel experimental model of giant cell myocarditis by immunizing Lewis rats with cardiac myosin in complete Freund’s adjuvant (CFA).14–16 The myocarditis was characterized by enlargement of the heart, pericardial effusion, discoloration of the cardiac surface, extensive myo-
cardial necrosis, and the frequent appearance of multinucleated giant cells in the lesions.

In this study, we attempted to clarify the origin of multinucleated giant cells in myocarditis by using our experimental model. The factors indispensible to the generation of giant cell myocarditis were also examined.

Materials and Methods

Animals

Female Lewis rats were purchased from Charles River Japan Inc., Atsugi, Japan, and maintained at the Facilities for Comparative Medicine and Animal Experimentation, Niigata University School of Medicine, Niigata, Japan.

Antigen

Purified cardiac myosin was used as an antigen. Cardiac myosin was prepared from the ventricular muscle of human hearts by a procedure previously described.16 Human hearts were obtained at autopsy from patients who had died of malignancy and had no history of myocarditis or congestive heart failure. No significant abnormalities were detected in the sample hearts.

Immunization

Cardiac myosin was dissolved in a solution of 0.3 M KCl and 0.2 M phosphate-buffered saline (PBS) at a concentration of 5 mg/ml. Female Lewis rats (7–8 weeks old) were injected in the footpads with 0.5 mg cardiac myosin in an equal volume of CFA containing 1 mg/ml of Mycobacterium tuberculosis. Rats were immunized again after 7 days.

In the next experiment, cardiac myosin was dissolved at various concentrations (i.e., 0.1, 0.35, 1.0, 3.5, and 10.0 mg/ml). Rats were immunized twice with 0.1 ml of the indicated antigen solution in an equal volume of CFA.

Adjuvants

The effects of immunoadjuvants on the induction of experimental giant cell myocarditis was investigated using conventional CFA, incomplete Freund’s adjuvant (IFA), CFA supplemented with M. tuberculosis H37Ra (Difco Laboratories, Detroit, Mich.) at a concentration of 11 mg/ml (CFA+H37Ra), and Bordetella pertussis vaccine (2×10⁷/ml) (Nakarai Chemical, Kyoto, Japan). Rats were divided into six groups. Rats of each group were immunized twice with 1.0 mg cardiac myosin mixed with one of these different adjuvants. B. pertussis vaccine was injected intravenously on days 1 and 3. Effects of administration of the antigen with Freund’s adjuvant alone and that of combined Freund’s adjuvants and B. pertussis were examined.

Histopathology

All rats were killed under ether anesthesia. Hearts were removed immediately after killing, and part of the ventricular muscle was frozen in liquid nitrogen for immunohistochemical study. The remainder was fixed in 10% formalin and embedded in paraffin. Several transverse sections were cut from paraffin-embedded samples and stained with hematoxylin and eosin. The macroscopic and microscopic findings of myocarditis were graded according to the following two scoring systems. Macroscopic findings were graded into three categories: 0, normal; 1, the presence of a focal discolored area; and 2, the presence of multiple or diffuse discolored areas on the cardiac surface. Microscopic findings were graded as follows: 0, normal; 1, the presence of a few small lesions in a single section not exceeding 0.25 mm² in size; 2, the presence of multiple small lesions or a few moderately sized lesions not exceeding 6.25 mm²; and 3, the presence of multiple moderately sized lesions or more larger lesions.

Monoclonal Antibodies

To investigate the character of multinucleated giant cells in experimental autoimmune myocarditis, immunohistochemical studies were carried out using various monoclonal antibodies: W3/25 (Serotec Inc., Oxford, UK), a marker for helper T cells and some macrophages; OX8 (Serotec), a marker for cytotoxic suppressor T cells; OX19 (Serotec), a marker for pan-T cells; OX33 (Serotec), a marker for B cells; OX42 (Serotec) and ED1 (Serotec), markers for macrophages; murine anti-desmin monoclonal antibody (Sigma Chemical Co., St. Louis, Mo.); and HHF35 (Enzo Biochem), antibody against skeletal muscle actin.

Immunohistochemistry

Hearts of the rats were snap-frozen in OCT compound (Miles Inc., Elkhart, Ind.). Sequential 5-μm-thick frozen sections were cut in a cryostat and fixed in ether for 10 minutes. After the sections were washed in PBS, they were incubated with 20% normal goat serum (TAGO Inc., Burlingame, Calif.) in PBS for 30 minutes. Monoclonal antibodies diluted to 1:200 (W3/25, OX8, OX19, OX42, and ED1), 1:100 (OX33), 1:20 (anti-desmin), and 1:1 (HHF35) were dropped onto the sections and incubated for 45 minutes. Slides were rinsed in PBS and incubated for 30 minutes with biotinylated goat anti-mouse immunoglobulin G (Amersham, Amersham Place, UK) diluted to 1:100 with 20% normal Lewis rat serum in PBS and followed by incubation for 45 minutes with horseradish peroxidase–labeled streptavidin (Amersham) diluted to 1:200. Reaction products were visualized with 0.05% diaminobenzidine and 0.03% H₂O₂ until a distinct color product was microscopically detected with minimal background staining. Sections were then counterstained with hematoxylin.

Adoptive Transfer

Lewis rats were immunized with human cardiac myosin fraction in CFA+H37Ra, followed by injection with B. pertussis. Spleens were removed, and a single-cell suspension was prepared by passing the spleens through a stainless-steel mesh screen. Spleen cells were
suspended at a concentration of 2×10⁶ cells/ml in RPMI 1640 supplemented with 10% fetal calf serum, 1% sodium pyruvate, 1% nonessential amino acids (GIBCO Laboratories, Grand Island, N.Y.), 0.1 mg/ml kanamycin (Meiji Seika, Tokyo, Japan), and 5×10⁻⁵ M 2-mercaptoethanol and were cultured for 3 days in the presence of 1 µg/ml concanavalin A. Cultured spleen cells were injected intravenously into naive syngeneic rats. Recipient rats were killed on days 11 and 20 for histological examination.

Statistics
The counts of multinucleated giant cells are expressed as mean±1 SD.

Results
Pathology and Clinical Course of Experimental Autoimmune Myocarditis
The onset of experimental autoimmune myocarditis and the appearance of multinucleated giant cells are summarized in Table 1. By the third week, all rats immunized with cardiac myosin showed ruffled fur and were unable to move. Hearts of the rats killed on days 7 and 14 showed no evidence of myocarditis. Two of four rats examined on day 16 had a large amount of pericardial effusion and cardiac enlargement. There were areas of discoloration on the cardiac surface. All 30 rats killed after day 18 had severe myocarditis. Pericardial effusion was observed in the rats examined between days 16 and 28. Histological investigation revealed extensive myocardial necrosis and cell infiltrations composed of lymphocytes, neutrophils, and macrophages. Calcification was not observed. Multinucleated giant cells were observed in one of four rats killed on day 16, two of five on day 18, two of eight on day 21, four of eight on day 28, and two of four on day 35. On day 42, cell infiltration and multinucleated giant cells disappeared and inflammatory changes were replaced by fibrosis. Multinucleated giant cells had five to 20 large and ovoid nuclei arranged in a horseshoe shape or circle (Figure 1). The majority of giant cells had round and slightly basophilic cytoplasm. A few rod-shaped giant cells lying in parallel with the muscle fibers were also noticed (Figure 2).

Immunohistochemical Analysis of Giant Cells
W3/25-positive cells were scattered in the inflammatory focus. OX8-positive cells were very few in number in this myocarditis during the peak inflammatory phase. B cells were not detected in the lesions. Multinucleated giant cells were not stained with the above monoclonal antibodies specific for lymphocytes (Figure 3). In this experimental autoimmune myocarditis, inflammatory cells were predominantly composed of OX42-positive cells. Multinucleated giant cells were also strongly stained with both OX42 and ED1, markers for macrophages (Figure 4). Although intact and degenerative muscle fibers were strongly stained with both anti-desmin antibody and HHF35, multinucleated giant cells were completely negative for these antibodies (Figure 5).

Effect of Dose of the Antigen on Giant Cell Formation
We next investigated how much antigen was necessary for the formation of giant cells. As shown in Table 2, rats immunized with 100 µg cardiac myosin showed only histological evidence of myocarditis without the presence of giant cells. Macroscopic changes, such as pericardial effusion, cardiac enlargement, and discoloration, were not detected in this group. The rats immunized with 350 µg antigen showed severe myocarditis, and many giant cells were detected in the lesions. The rats injected with 1 mg cardiac myosin had more severe myocarditis and a greater number of giant cells.

Immunoadjuvants and Giant Cell Myocarditis
The effects of immunoadjuvants for the induction of autoimmune myocarditis were investigated (Table 3). All the rats sensitized with the antigen in CFA or CFA+H37Ra developed severe myocarditis, and most of them showed multinucleated giant cells in the lesions. Rats immunized with cardiac myosin in IFA followed by the injection of B. pertussis also showed severe myocarditis with macroscopic findings, but giant cells were not present in the lesions. In the rats sensitized with IFA alone, no signs of myocarditis were detected.

Adaptive Transfer of Giant Cell Myocarditis
Seven of the nine rats injected with sensitized spleen cells that had been cultured for 3 days in the presence of concanavalin A demonstrated myocarditis (Table 4). Multinucleated giant cells were observed in two of nine rats, but the frequency of giant cells was lower than in those with actively induced myocarditis.

<table>
<thead>
<tr>
<th>Table 1. Clinical Course and Appearance of Giant Cells in Experimental Autoimmune Myocarditis</th>
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<tbody>
<tr>
<td>Day of</td>
</tr>
<tr>
<td>examination</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>7</td>
</tr>
<tr>
<td>14</td>
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<td>16</td>
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<td>18</td>
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<td>21</td>
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<tr>
<td>28</td>
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<tr>
<td>35</td>
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<tr>
<td>42</td>
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</table>

All rats were immunized with 0.5 mg cardiac myosin in complete Freund's adjuvant and not followed by Bordetella pertussis injection. GCM, giant cell myocarditis; GC, giant cells.

*Scores are expressed as the mean for all rats.
†Mean counts of giant cells in one section. Two sections per rat with giant cell myocarditis were examined.
‡Inflammatory infiltrates were partially replaced by fibrosis, but the extent of lesions was broad.
Discussion

Giant cell myocarditis is a disease entity defined by its pathology and not by its etiology. The conditions related to the generation of giant cell myocarditis remain unknown. In addition, there has been no experimental model for giant cell myocarditis to confirm its pathogenesis. In the present study, we have demonstrated a novel experimental autoimmune giant cell myocarditis. This experimental myocarditis is one of the organ-specific autoimmune diseases caused by delayed-type hypersensitivity.

This model is closely similar to human giant cell myocarditis in its morphological features and the severity of inflammation in the heart. Otherwise, this animal model is apparently different from the human disease in its initiation process, namely, immunization with the antigen in immunoadjuvants. The process never does exist in humans, and other events such as viral infection or toxic exposure may act as a similar process, namely, strong activation of antigen-reactive lymphocytes and enhancement of immune responsiveness of the host. There are also some incomparable features between this model and the disease in humans, for example, the etiology, the features of susceptible individuals, and precise clinical course, because the disease in humans has not been fully understood. Although there are some different features between this animal model and human giant cell myocarditis, this model provides important information regarding the formation of multinucleated giant cells during acute myocarditis.

Multinucleated giant cells in human acute myocarditis have long been considered to be of myogenic origin. Actually, some supporting evidence has been demonstrated. The giant cells occasionally show rod-shaped cytoplasm that is found lying parallel to the surrounding muscle fibers. A direct connection of plasma membrane between giant cells and muscle fibers can also be observed.
Light and electron microscopic studies have revealed the presence of various components of muscle fibers, such as myofibrils, intercalated disks, and lipofuscin granules, in the cytoplasm of giant cells. On the other hand, Theaker et al. and Hales et al. reported evidence for macrophage-derived giant cells by using immunohistochemical staining. The multinucleated giant cells described in their reports were stained with anti-macrophage antibody but not with an antibody against desmin intermediate filaments. Both types of giant cells, of myocyte-like and macrophage-like appearance, were observed in this experimental autoimmune myocarditis in light microscopic investigation. Immunohistochemical studies, however, revealed that both types of giant cells were strongly stained with OX42 and ED1 (macrophage markers) and completely negative for an anti-desmin antibody (HHF35) and all the lymphocyte markers. Therefore, giant cells in this myocarditis seem to be derived from macrophages. We consider that myocyte-like giant cells observed in this model are a fusion of macrophages that are ingesting degenerated muscle fibers.

There may be two forms of giant cell myocarditis in humans: with macrophage-derived giant cells and with myocyte-derived giant cells. However, based on the above findings, we think it is unlikely that multinucleated giant cells in human myocarditis are derived only from myocytes. Since a cardiomyocyte generally has a single nucleus, excessive mitosis or cell fusion would be necessary for myocytes to form...

TABLE 3. Relation Between Adjuvants Used and Induction of Giant Cell Myocarditis

<table>
<thead>
<tr>
<th>Freund’s adjuvant</th>
<th>Bordetella pertussis</th>
<th>Pericardial effusion</th>
<th>Macroscopic score</th>
<th>Histological score</th>
<th>GCM (No. of GC*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFA + H37Ra</td>
<td>+</td>
<td>8</td>
<td>2.0</td>
<td>3.0</td>
<td>9 (31.1±15.6)/9/9</td>
</tr>
<tr>
<td>CFA + H37Ra</td>
<td>–</td>
<td>3</td>
<td>1.3</td>
<td>2.8</td>
<td>3 (12.7±16.6)/4/4</td>
</tr>
<tr>
<td>CFA</td>
<td>+</td>
<td>6</td>
<td>1.8</td>
<td>3.0</td>
<td>5 (25.7±31.6)/6/6</td>
</tr>
<tr>
<td>CFA</td>
<td>–</td>
<td>5</td>
<td>1.4</td>
<td>3.0</td>
<td>1 (3.5±5.0)/5/5</td>
</tr>
<tr>
<td>IFA</td>
<td>+</td>
<td>1</td>
<td>1.8</td>
<td>2.5</td>
<td>0/4/4</td>
</tr>
<tr>
<td>IFA</td>
<td>–</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0/0/3</td>
</tr>
</tbody>
</table>

Rats were immunized with 1.0 mg cardiac myosin. All rats were killed on day 21. GCM, giant cell myocarditis; GC, giant cell; CFA + H37Ra, complete Freund’s adjuvant supplemented with Mycobacterium tuberculosis H37Ra at a concentration of 11 mg/ml; IFA, incomplete Freund’s adjuvant.

*Mean number of giant cells in one section. Two sections per rat with giant cell myocarditis were counted.
multinucleated giant cells. However, mitosis or fusion of myocytes is unfamiliar. In diseases other than myocarditis, there have been several reports concerning the formation of multinucleated giant cells from macrophages and multinucleated giant cells are considered to be formed by cell fusion and not by cell division. Galindo demonstrated that alveolar macrophages from rabbits sensitized with H37Ra showed extensive development to multinucleated giant cells after incubation with heat-killed H37Ra. Galindo et al also demonstrated that macrophage fusion factors produced by antigen-stimulated lymph node cells play an important role in giant cell formation. McInnes and Rennick demonstrated that interleukin-4 induced in vitro formation of multinucleated giant cells by monocytes via cell fusion. Since this experimental myocarditis is an autoimmune disease that is generated by sensitized T cells, effector T cells may secrete various lymphokines, such as macrophage fusion factors or interleukin-4, in the lesions.

**TABLE 4. Frequency of Giant Cell Myocarditis in Adoptively Transferred Myocarditis**

<table>
<thead>
<tr>
<th>Transferred spleen cells</th>
<th>Macroscopic score</th>
<th>Histological score</th>
<th>GCM (No. of GC*) /diseased/total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 x 10^7</td>
<td>0.5</td>
<td>2.0</td>
<td>1 (1.5 ± 1.5)/2/4</td>
</tr>
<tr>
<td>2 x 10^7</td>
<td>1.0</td>
<td>2.0</td>
<td>1 (0.5 ± 0.5)/2/2</td>
</tr>
<tr>
<td>4 x 10^7</td>
<td>1.0</td>
<td>1.5</td>
<td>0/2/2</td>
</tr>
<tr>
<td>8 x 10^7</td>
<td>1.0</td>
<td>2.0</td>
<td>0/1/1</td>
</tr>
</tbody>
</table>

GCM, giant cell myocarditis; GC, giant cells.

*Mean counts of giant cells in one section. Two sections per rat with giant cell myocarditis were examined.*
In human giant cell myocarditis, a similar pathogenesis may be operating.

This myocarditis has a monophasic clinical course. The onset of the disease was about 16 days after the first immunization, and the fulminant phase continued for 2 weeks. The myocarditis then subsided by the sixth week. Multinucleated giant cells were observed during a considerably short period that corresponded with the fulminant phase of the disease. By the sixth week, giant cells were absent from the lesions. Therefore, the appearance of multinucleated giant cells in myocarditis seems to be closely related to the phase of myocarditis. Clinically, the onset of giant cell myocarditis is unclear, and sequential histological observation of the disease is quite difficult. Therefore, it is uncertain whether the appearance of multinucleated giant cells in the lesions is a continuous finding during the clinical course of the disease or only a temporary finding. Our study strongly suggests that the appearance of giant cells in myocarditis is a temporary finding.

In this study, we have also demonstrated that the generation of giant cell myocarditis requires a large amount of immunizing antigen. Because the severity of this myocarditis is related to the amount of antigen, the difference between giant cell and non-giant cell myocarditis may hinge on the severity of the disease. The functional capacity of a single macrophage to remove foreign materials is limited for the reason that the amount of enzymes for digestion is finite. It has been demonstrated that multinucleated giant cells have a strong activity of ingestion and digestion, the same as, or more than, macrophages. Therefore, the formation of giant cells may be useful in severe lesions that have extremely large amounts of foreign materials or necrotic debris.

In the liver and lungs of experimental animals, immunoadjuvants, especially those containing dead bacterial components, have been proven to induce focal granulomatous lesions. Therefore, one can speculate that the formation of giant cells depends on the chemical side effects of immunoadjuvants. We think such a possibility is less likely because of the following reasons. The effects of immunoadjuvants on the heart were investigated by Laufer et al in 1966. They demonstrated cardiac lesions in rats injected with CFA weekly for 6 weeks. About 11% of the rats showed focal mononuclear cell infiltration in the hearts, but neither severe myocarditis nor giant cell myocarditis was observed. Much more CFA and dead mycobacteria were used in Laufer’s experiments than in our experiments. Moreover, we demonstrated that no myocardial lesions were induced in rats immunized with nonmyocarditogenic antigen in CFA + H37Ra. Therefore, the mechanism that generated myocarditis in our study must not be of a simple chemical stimulation by the adjuvants. Rather, autoimmune processes against cardiac myosin seem to play an important role in the formation of giant cells.

Figure 4. Panel A: Multinucleated giant cells are strongly stained with OX42, a marker for macrophages (arrowheads). Inflammatory infiltrates are predominantly composed of OX42-positive cells. Panel B: Multinucleated giant cells are also stained with ED1, a marker for rat macrophages. The arrowhead indicates a giant cell stained with this antibody. Bars, 40 μm.
In this study, the rats sensitized with CFA and CFA+H37Ra showed giant cell myocarditis regardless of the use of *B. pertussis*. Rats immunized with cardiac myosin in IFA followed by an injection with *B. pertussis* showed severe non–giant cell myocarditis. No myocarditis was observed in rats sensitized with IFA without a *B. pertussis* injection. Accordingly, immunoadjuvants seem to determine the character of the disease: whether it is giant cell or non–giant cell myocarditis. The effects of CFA on the immune system have not been fully understood. The mineral oil contained in Freund’s adjuvant functions as a depot for the antigen. The muramyl dipeptidase contained in dead mycobacteria has various functions as an “immunostimulator”: it enhances helper T-cell function, stimulates T-cell proliferation, and activates Ia expression on macrophages. To induce giant cell myocarditis, dead mycobacteria is an essential component in the adjuvant. Therefore, it seems that acute myocarditis, dead mycobacteria is an essential component in the adjuvant. Therefore, it seems that acute myocarditis, under specific conditions of strongly activated macrophages or T cells, shows the pathological features of giant cell myocarditis.

Not only autoimmune myocarditis but also the appearance of multinucleated giant cells was transferable into naive syngeneic rats with concanavalin A–activated spleen cells. Because the spleen cells were washed several times before the transfer, neither antigen, adjuvants, nor pathogen may play a role in the development of myocarditis or in the formation of giant cells in transferred myocarditis. From this study, concanavalin A–activated spleen cells, namely, T cells, played an important role in the generation of giant cell myocarditis. However, it should be elucidated in future studies whether the activated T cells themselves or the reactions of host cells after the injection of concanavalin A–activated T cells are essential in the formation of multinucleated giant cells.

We have investigated the origin of multinucleated giant cells in experimental autoimmune myocarditis and have discussed the conditions related to the generation of giant cell myocarditis. Macrophage–predominant inflammation and the production of macrophage fusion factors seem to be essential for the generation of giant cell myocarditis. These conditions are able to occur in the inflammatory lesions of delayed-type hypersensitivity. In clinical cases of giant cell myocarditis, delayed-type hypersensitivity should be considered, even if the antigen is unknown.

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References


KEY WORDS • myocarditis • giant cell • cardiac myosin • macrophage
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