Histopathological Findings of Acute and Convalescent Myocarditis Obtained by Serial Endomyocardial Biopsy

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Serial endomyocardial biopsy findings in 20 cases with acute myocarditis of possible viral origin were analyzed. The histopathological findings were analyzed during the acute (0–10 days after the onset: 8.3 ± 1.9 days; 6 cases), the subacute (11–21 days: 18.2 ± 2.2 days, 6 cases), and the convalescent stages (22–167 days: 54.5 ± 45.4 days; 8 cases). The incidence and severity of various changes of the cardiac myocytes and interstitial changes were analyzed and compared at each stage of the disease. In the acute stage, interstitial cell infiltrations were composed of fibroblasts, macrophages and lymphocytes, taken out in descending order. In the convalescent stage, interstitial cell infiltration showed a marked increase and was replaced by fibrocytes. In the subacute stage, transitional changes between the acute and convalescent stages were observable. A controlled myocardium in 21 cases with myocardial infarction which were compared at various stages revealed that in the acute stage, neutrophils were most prominent, and in the subacute and convalescent stages, macrophages were most prominent and plasma cells were most often observed.

The time course changes of the histopathological findings in acute myocarditis were as follows: In the acute stage, 1) Interstitial cell infiltration which is composed of fibroblasts, macrophages and lymphocytes, 2) fragmentation of the muscle bundles, 3) myocytolytic changes, 4) swelling and scarcity of the cytoplasm and swelling of nuclei, 5) variation in size of the myocytes, 6) disarrangement of the muscle bundles, 7) interstitial edema, 8) increased glycogen deposition in the myocytes, 9) abnormal branching of the myocytes, and 10) interstitial fibrosis were observable. In the convalescent stage, most of the above described findings were still observable except for the myocytolytic change, swelling of myocytes and interstitial edema. In the subacute and convalescent stages, an increase in abnormal branching, an increase in double nuclei in the myocytes, and nuclear degeneration were observable. Further control study comparing the change in the convalescent stage of myocarditis and the myocardial changes in cases with chronic right ventricular overload in 58 cases revealed that in the former, fragmentation of the muscle bundles, abnormal branching, size variation, glycogen deposition, and large mononuclear cell infiltrations were significantly more frequent.

It is concluded that serial endomyocardial biopsy is useful in assessing the time-course changes of the myocardium in myocarditis.

Key Words:

Viral myocarditis
Endomyocardial biopsy
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THE diagnosis of acute myocarditis of possible viral origin can be made with the help of a careful study of the history of flu syndrome, electrocardiographic changes and a rise of the neutralizing titer in the serum!¹⁻³ However, none of the diagnostic elements permit a conclusive diagnosis of this disease. We have been performing endomyocardial biopsies in cases with myocarditis and have come to the conclusion that the histopathological diagnosis of acute myocarditis and postmyocarditic change can be made when the biopsy is performed within 10 days after the onset of the disease.² This report is a histopathological analysis of 10 cases with acute myocarditis on whom serial endomyocardial biopsies were performed. After the detailed analysis of each finding, a proposal for the diagnostic criteria of acute myocarditis and postmyocarditic change is made.

MATERIALS AND METHOD

The biopsies were performed in 10 cases with acute myocarditis of possible viral origin except for 1 case, in which serial endomyocardial biopsies was performed (Group 1).²⁻⁵ The breakdown of the 10 cases has been reported previously.^{2,5} A histopathological examination of the biopsied specimens was routinely made. The findings which were analyzed consisted of 23 histopathological changes and the method of interpretation and grading of the findings has also been previously reported.^{6,7} The disease process was divided into 3 stages; acute, subacute and convalescent stages. For the control study, myocardial specimens from 21 cases with myocardial infarction in the acute and convalescent stages were taken either at the time of cardiac surgery or autopsy (Group 2), (See Table I). For the comparison of the histopathological

changes, the specimens from cases with myocardial infarction were analyzed according to the stage classification as it applied to myocarditis. The reason why we selected the cases with myocardial infarction was to compare the cell components of the interstitium which seemed to have occurred to react to the necrotic heart muscle cells.

Another control group (Group 3) was made in order to discover the changes which occur as for example secondarily occurring myocardial hypertrophy in various heart diseases.⁸ The disease group consisted of 25 cases with valvular heart disease, 28 cases with congenital heart disease, 3 cases with primary pulmonary hypertension, 1 case with isolated tricuspid regurgitation and 1 case with cor pulmonale.⁸ Interstitial cell differentiations were made with the help of oil immersion and 50 cells randomly selected were classified according to the characteristic cell morphology employing hematoxylin-eosin, Mallory's, elastica Van-Gieson's and PAS (Periodic Acid Schiff) stain.⁹⁻¹² In order to justify our histopathological diagnosis, ultrastructural analysis was performed on the same biopsied specimen.¹³

Statistical analysis was made using Student's t-test.

RESULTS

In Fig. 1, a breakdown of the cell components which were observed in the various stages is shown. In order to compare the interstitial cell components in cases with acute myocarditis and in myocardial infarction, cells were differentiated and the incidence of the appearance of the cell constituents is shown in Fig. 2. It can be seen that in the acute stage of myocarditis, fibroblasts are most frequently observed and in the conva-

TABLE I FOR THE CONTROL STUDY, MYOCARDIAL SPECIMENS FROM CASES WITH MYOCARDIAL INFARCTION WERE TAKEN EITHER AT THE TIME OF HEART SURGERY OR WITHIN 3 HOURS AFTER DEATH OF THE PATIENT

Disease	Stage		
	Acute (0-10 days)	Subacute (11-21 days)	Convalescent (22-167 days)
Myocarditis (n = 20)	n = 6 (biopsy)	n = 6 (biopsy)	n = 8 (biopsy)
Myocardial infarction (n = 21)	n = 8 surgery 1 necropsy 7	n = 5 surgery 1 necropsy 4	n = 8 surgery 7 necropsy 1

Fig.1. Examples of interstitial cell infiltration in cases with myocarditis and myocardial infarction (shown by arrows).

A: Large irregularly-shaped fibroblasts in myocarditis. Ninth day after the onset; B: Lymphocytes. Seventh day after the onset; C: Macrophages. Twentieth day after the onset of myocarditis; D: Mast cells seen on the 17th day of myocarditis; E: Fibrocytes seen on the 167th day of myocarditis; F: Macrophages, in which phagocytosed erythrocytes are seen, are prominent in the infarcted myocardium (fourth day after the onset); G: Plasma cells in the infarcted myocardium taken on the 25th day after the onset.

lescent stage, fibrocytes are prominent.

On the contrary, in acute myocardial infarction, neutrophils were most prominently observed. Table II shows the incidence of cellular constituents in myocarditis and myocardial infarction. It can be seen that in acute myocarditis, fibroblasts, macrophages, lymphocytes are prominent and in the subacute stage, fibroblasts became more prominent. In the convalescent stage, fibrocytes became most prominent. In myocardial infarction, neutrophils were most prominent in the acute stage. In the subacute and convalescent stages, macrophages were most prominent. It should be noted that, in the convalescent stage, plasma cells sometimes appeared frequent.

Figure 3 illustrates the percent incidence of various histopathological findings in the acute,

subacute, and convalescent stages. The figure shows that in the convalescent stage, interstitial edema has decreased. Small round cell (lymphocytes and plasma cells) infiltration also has decreased (Fig. 2). Swelling of nuclei and myocytolysis have also decreased during the course of the disease. Hypertrophy of the myocytes, was observed and nuclear changes such as pyknosis, enlargement and deformities were also more prominent in the subacute and convalescent stages. Furthermore, an increase in the frequency of double nuclei was noted. Figure 5 illustrates a comparison between the findings in the convalescent stage of myocarditis and the myocardial changes in chronically overloaded right ventricular myocardium. In 9 histopathological parameters, the statistical difference of the frequency of the findings are noted. It can

Fig. 2. Cellular constituents in myocarditis and myocardial infarction. Percent incidence of the cell components are shown according to the stage differentiation of the disease. Fifty interstitial cells were classified according to the incidence of the appearance of the cells.

TABLE II PERCENT INCIDENCE OF CELLULAR CONSTITUENTS IN MYOCARDITIS AND MYOCARDIAL INFARCTION. THE NUMBERS ARE EXPRESSED WITH THE MEAN VALUE AND STANDARD DEVIATION

Disease	Stage					
	Acute (0–10 days)		Subacute (11–21 days)		Convalescent (22–167 days)	
Myocarditis (n = 20)		n = 6		n = 6		n = 8
	Fibroblast	35.3 ± 3.5	Fibroblast	29.2 ± 6.5	Fibrocyte	54.4 ± 7.2
	Macrophage	25.3 ± 3.0	Macrophage	20.3 ± 5.4	Macrophage	13.1 ± 5.2
	Lymphocyte	16.9 ± 6.0	Fibrocyte	17.5 ± 8.7	Fibroblast	11.7 ± 4.0
	Fibrocyte	8.9 ± 4.8	Lymphocyte	16.7 ± 9.9	Lymphocyte	8.1 ± 6.0
	Neutrophil	0	Plasma Cell	2.0 ± 2.7	Plasma Cell	1.3 ± 1.7
Myocardial infarction (n = 21)		n = 8		n = 5		n = 8
	Neutrophil	40.0 ± 7.8	Macrophage	34.7 ± 1.4	Macrophage	33.7 ± 8.2
	Macrophage	26.5 ± 7.5	Fibrocyte	17.0 ± 5.9	Fibrocyte	21.9 ± 7.9
	Fibroblast	16.2 ± 2.9	Fibroblast	15.0 ± 6.2	Fibroblast	16.2 ± 9.1
	Fibrocyte	6.5 ± 4.3	Lymphocyte	13.7 ± 5.1	Lymphocyte	10.2 ± 5.4
	Lymphocyte	5.8 ± 3.1	Plasma Cell	8.3 ± 4.3	Plasma Cell	10.0 ± 9.3

Fig.3. Constituents of the severity of myocardial degeneration in a biopsied right ventricular myocardium in 3 stages of myocarditis.

Fig.4. Incidence of the severity of myocardial hypertrophy, abnormalities of muscle arrangement, degeneration of myocytes and interstitial fibrosis in the biopsied right ventricular myocardium in 3 stages of myocarditis.

Fig.5. Histopathological findings in myocarditis in the convalescent stage and in chronic right ventricular overload (CRVO). In this figure, comparison of the incidence of the significant findings are shown.

be seen that abnormal branching, fragmentation of muscle bundles, increased glycogen deposition and the increase of large mononuclear cell infiltration are most prominent.

DISCUSSION

A series of studies of histopathological diagnosis of viral myocarditis has made progress in assessing this disease with the help of long-standing experience in interpreting biopsied specimen over the past 15 years.¹⁴ Also, the results of this study may lead us to propose diagnostic criteria for acute and postmyocarditic changes.^{2,15}

The histopathological characteristics of acute myocarditis are summarized as follows:

- 1) Interstitial cell infiltration which is composed of polymorphous fibroblasts, macrophages and lymphocytes.
- 2) Fragmentation of the muscle bundles.
- 3) Various forms of myocardial degeneration which are characterized by progressing myocytolysis and disappearance.
- 4) Swelling of the sarcoplasm and nuclei as well as scarcity of the myofibrils of myocytes.
- 5) Variation in size of myocytes.
- 6) Disarrangement of the muscle bundles.
- 7) Interstitial edema.
- 8) Increase in glycogen deposition in myocytes.
- 9) Increase of myocyte branching.
- 10) Interstitial fibrosis.

The postmyocarditic changes are:

- 1) Various changes which are observed in acute myocarditis still exist, however, myocytolysis, swelling of sarcoplasm of myocytes and interstitial edema become unobservable.
- 2) Decrease in large mononuclear cell infiltration in the interstitium and increase in the replacement of fibrocytes.
- 3) Size of the myocytes increase showing hypertrophy which is associated with pyknosis, enlargement and deformities of the nuclei.
- 4) Increase in the incidence of double nuclei of the myocytes.
- 5) Increase of abnormal branching of myocytes.
- 6) Increase in interstitial fibrosis.

The value of endomyocardial biopsy in the clinical setting has been demonstrated by our previous study²⁻⁵. The authors presume that the proper interpretation of the histopathological findings in cases with acute myocarditis are

considered to be both valuable and superior to the determination of titration for the viral infection in the patients' serum. The authors note that Billingham¹⁶ of Stanford University, where cardiac transplantations have been continuously carried out, is of the opinion that the biopsy findings yield reliable diagnostic elements when assessing the rejection phenomena inside the heart muscle. Hiroe¹⁷ of our group has made clear that the proper interpretation of the biopsy findings correlate well with the hemodynamic characteristics in the acute, subacute and convalescent stages. He employed our histopathological criteria of the grading of acute myocarditic change.

Shibata¹⁸ of our group has observed a positive correlation between the course of electrocardiographic and vectorcardiographic findings in the same cases as in this report. Take¹⁵ has analyzed biopsied specimens in a large number of cases (560 cases) in which the results indicated that the postmyocarditic change (PMC) was observed in 15% of DCM cases and that 8.7% of the arrhythmia-conduction disturbance type of cardiomyopathy, and in only 1.7% of HCM. This data was supported by the presence of the flu-syndrome in about 30% of the cases where the PMC was assessed. Hasumi¹⁹ has further analyzed cases with A-V block and fascicular block and found that in 40% of the biopsied cases, significant pathology was found and among them, 50% of those cases with significant pathology, the PMC was observed. It is concluded that the proper histopathological analysis and gradings of the severity of the disease can be applied to the clinical evaluation in myocarditis and allied disease groups.

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