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ORIGINAL ARTICLE

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Ultrastructural and immunohistochemical analysis of biopsy-proven chronic active mycocarditis with numerous clusters of lymphocytes

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Abstract A 60-year-old women was admitted to our hospital with deteriorating congestive heart failure. Although the diagnosis of active myocarditis was confirmed by right ventricular endomyocardial biopsy, this patient died of refractory heart failure during corticosteroid treatment. Numerous lymphocytic clusters were observed microscopically in the heart at autopsy. Most of the infiltrating cells in the clusters were positive for CD 8, HAM 56 or MHC class 2 antigen; few cells were positive for CD 56. Expression of perforin was found in some of the infiltrating cells. Electron microscopic examination revealed small lymphocytes adhering to the surface of injured cardiac myocytes. Close contact of these lymphocytes to macrophages was shown in the clusters. ICAM-1 and MHC class 1 antigens were strongly expressed in the cardiac tissue. These results indicate that cytotoxic T lymphocyte-mediated cytotoxicity had continued to operate during immunosuppressive therapy. Corticosteroids may not be suitable for the treatment of chronic active myocarditis when persistent expression of ICAM-1 is observed.

Key words Congestive heart failure · Corticosteroids · Chronic active myocarditis

Introduction

Recently Lieberman et al. proposed a new classification for myocarditis based on their clinical and histopathological experience [10]. Chronic active myocarditis was characterized by an indistinct onset, progressive deterioration and the development of an end-stage dilated cardiomyopathy. Chronic myocarditis has since been discussed as a clinicopathological entity characterized by the existence of lymphocytic clusters, and most cases have been diagnosed at autopsy [9, 15, 16]. Various studies using murine models of viral acute myocarditis have already contributed a good deal of information on the pathogenesis of and therapeutic approaches to this disease [5, 7, 13, 17, 18]. However, the exact mechanism of myocardial cell damage and the signal of ongoing inflammation in chronic active myocarditis remains uncertain, because of its rare clinical conditions and the lack of appropriate animal models.

We describe a rare case of chronic active myocarditis with numerous clusters of lymphocytes confirmed by right ventricular endomyocardial biopsy. Immunohistochemical and ultrastructural investigations were performed to clarify the mechanism of myocardial injury in human chronic active myocarditis. Corticosteroid treatment of this disease is also discussed.

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Clinical history

A 60-year-old woman without preceding episodes of acute myocarditis had experienced insidious, but progressive epigastric discomfort, fatigue and dyspnoea since November 1992. Because the signs and symptoms of congestive heart failure gradually worsened she was admitted to our department for further examination and treatment in March 1993.

On physical examination, her blood pressure was 98/62 mm Hg, with a heart rate of 86 beats/min. There was engorgement of the jugular vein and hepatojugular reflux, but no peripheral oedema. A grade 2/6 holosystolic murmur and the third heart sound were heard. The liver measured one finger breadth below the costal margin on palpation. Her laboratory findings showed a normal blood count and biochemistry.

The chest X-ray film on admission demonstrated cardiomegaly with a cardiothoracic ratio of 62%. An electrocardiogram taken on admission revealed low voltage in the limb leads, R wave regression in the precordial leads and left axis deviation. Results of the pressure study showed the following: pulmonary capillary wedge pressure 22 mmHg, mean pulmonary arterial pressure 30 mmHg, right atrial pressure 12 mmHg, and left ventricular end-diastolic pressure 24 mmHg. The cardiac index measured by the thermodilution method was 1.82 l/min per mm^2. The echocardiogram and the left ventriculogram revealed dilated cardiomyopathy-like conditions with an ejection fraction of 20% because of normal coronary artery, but the patient had abnormal accumulations of gallium-67 and technetium-99m pyrophosphate in the myocardium.

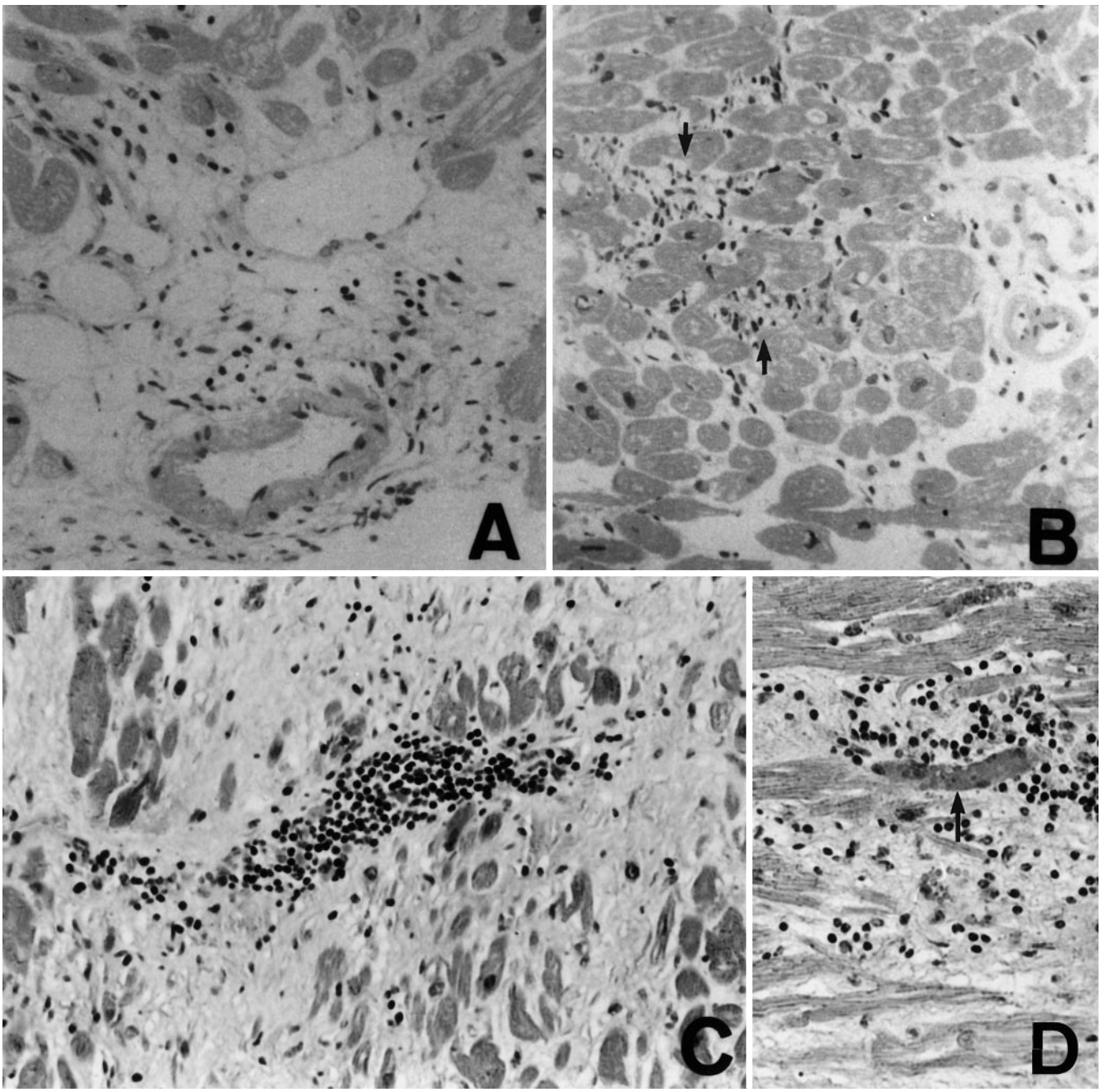

Fig. 1A, B Photomicrographs of the specimens of heart tissue obtained by endomyocardial biopsy, showing inflammatory infiltrates **A** around the small vessel and **B** adjacent to degenerative myocytes (*arrows*). **C**, **D** Photomicrographs of the specimens obtained from the autopsied heart, demonstrating **C** lymphocytic clusters with severe interstitial fibrosis and **D** the destruction (eosinophilic degeneration, *arrow*) of cardiac myocytes. Hematoxylin-eosin, original magnification ×200

The diagnosis of active myocarditis was confirmed according to the Dallas criteria [1] by right ventricular endomyocardial biopsy. Biopsy specimens showed interstitial infiltration of mononuclear cells around the vessels (Fig. 1B) and adjacent to the degenerative myocytes (Fig. 1A). In spite of the administration of corticosteroids, including their use for pulse therapy, the signs and symptoms of congestive heart failure worsened rapidly. Ultimately, the patient died of multiple organ failure in June 1993. The autopsy was performed about 2 h after her death.

Materials and methods

Right and left ventricular tissues were fixed in 4% paraformaldehyde/0.1 M sodium phosphate for 6 h at 4° C. After washing in 0.1 M lysin/0.15 M sodium phosphate and 0.15 M sodium chloride for 1 h at 4° C, they were immersed in graded sucrose solution; 10% 15%, and 20% for 10 h each at 4° C [19]. This material was quick-frozen in O.C.T. compound (Miles, Elkhart, Indiana). Cryostat sections 6 µm thick were cut and collected on poly-L-lysin-coated glass slides. These slides were air-dried for about 60 min.

Fig. 2 Immunoperoxidase staining of the infiltrating cells in the autopsied heart. In the clusters, most of the infiltrating cells are positive for CD8, HAM56 or MHC class 2 antigens. Expression of perforin is found in some of the infiltrating cells around the clusters (*arrows*), but not in the clusters ▶

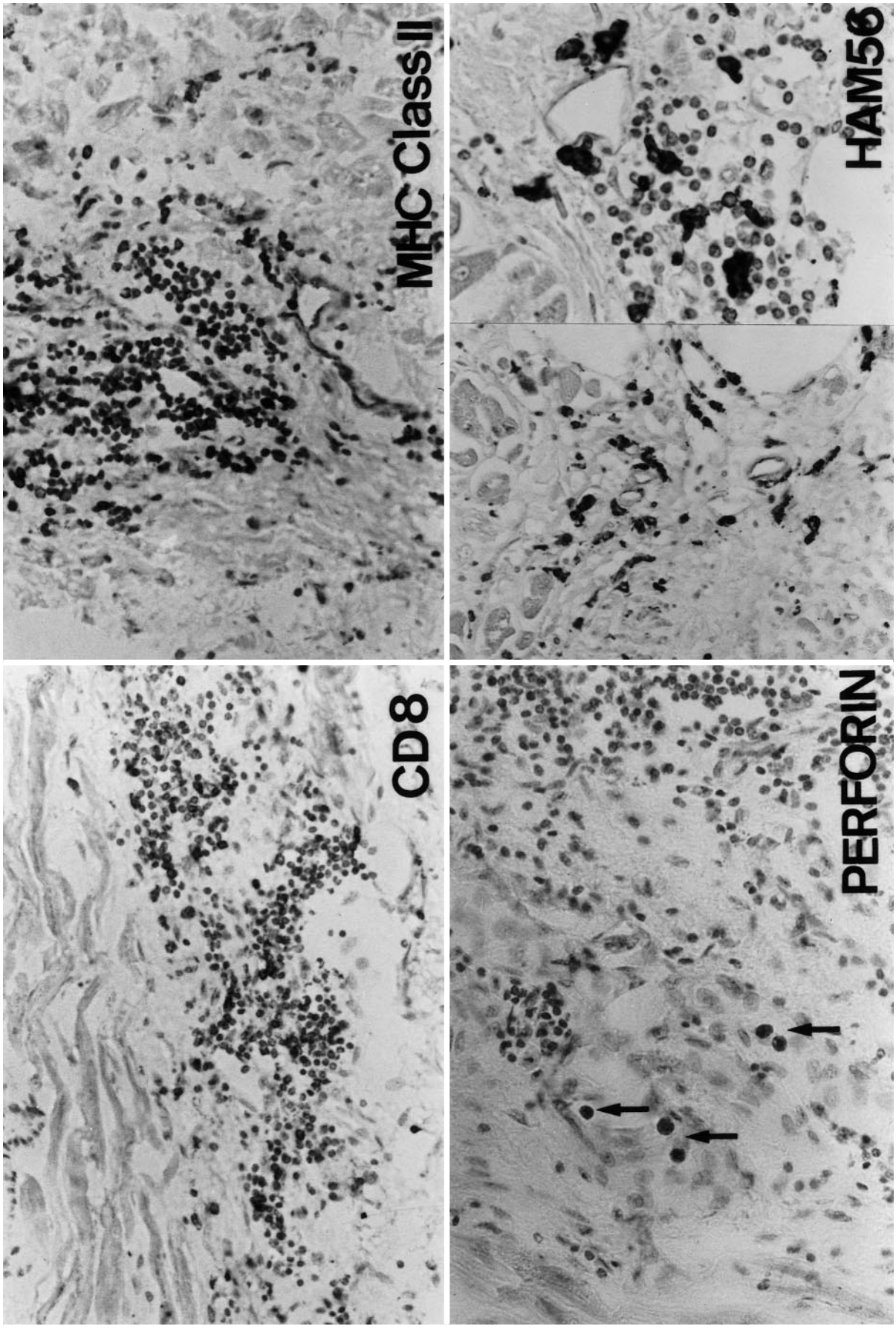
MHC Class II
HAM56
CD8
PERFORIN

Table 1 Primary antibodies (*MHC* major histocompatibility antigen, *ICAM* intercellular adhesion molecule, *VCAM* vascular cell adhesion molecule)

Antibodies	Clone	Dilution	Source
CD4	MT310	1:20	Dako
CD8	DK25	1:200	Dako
CD20	L26	1:100	Dako
CD45RO	UCHL1	1:100	Dako
CD56	T199	1:50	Dako
HAM56	HAM56	1:50	Dako
Perforin	IB4	1:100	Sumitomo
MHC class 1	W6/32	1:100	Dako
MHC class 2	CR3/43	1:100	Dako
ICAM-1	84H10	1:100	Immunotech
VCAM-1	1G11	1:100	Immunotech
E-selectin	1.2B6	1:100	Immunotech

The immunophenotype of the infiltrating cells and the expression of cell surface molecules were investigated by using a standard avidin-biotin immunoperoxidase technique. The sources, specificity, and working dilution of the primary antibodies used are listed in Table 1. Appropriate negative controls in which the primary antibody was replaced by PBS (0.02 mM, pH 7.6) were used for all immunostaining procedures.

Small heart tissue specimens obtained at biopsy and autopsy were fixed in 2.5% glutaraldehyde in phosphate buffer (pH 7.4), postfixed in 1% O_sO4, dehydrated and embedded in Epon 812 for electron microscopy. After observation of toluidine blue-stained 1-µm sections the representative area was trimmed. Ultrathin sections were stained with uranylacetate and lead citrate, and examined with an electron microscope (JEM 1200).

Pathological findings

The autopsied heart weighted 375 g. The main gross feature was moderate biventricular dilatation. Light microscopic examinations revealed extensive lymphocytic infiltration with myocyte damage and interstitial fibrosis especially in the middle layer of the left ventricular wall. Prominent clustered lymphocytic infiltration was also present throughout the interstium (Fig. 1C). Destruction of the cardiac myocytes caused by the inflammatory infiltrates was clearly seen (Fig. 1D).

Results of the immunohistochemical analysis of the infiltrating cells are shown in Fig. 2. In the clusters, most infiltrating cells were positive for CD45RO, CD8, HAM56 or MHC class 2 antigens, and a small number of CD4 positive cells were also detected. However, no CD56- or CD20-positive cells were seen in the clusters. Capillary endothelial cells were also stained with MHC class 2 antigens. HAM56-positive cells were frequently seen both in the interstium and in the clusters. Expression of perforin was found in some of the infiltrating cells around the clusters and near the injured myocytes, but not in the clusters.

Electron microscopic examinations were also performed (Fig. 3). Many lymphocytes in close contact with cardiac myocytes were observed. In the clusters, many lymphocytes were around the large and irregularly shaped cells, which were probably macrophages. Some of the lymphocytes were in contact with the macrophages and other lymphocytes. However, we could not find natural killer-like large granular lymphocytes containing electron-dense granules in the cytoplasm.

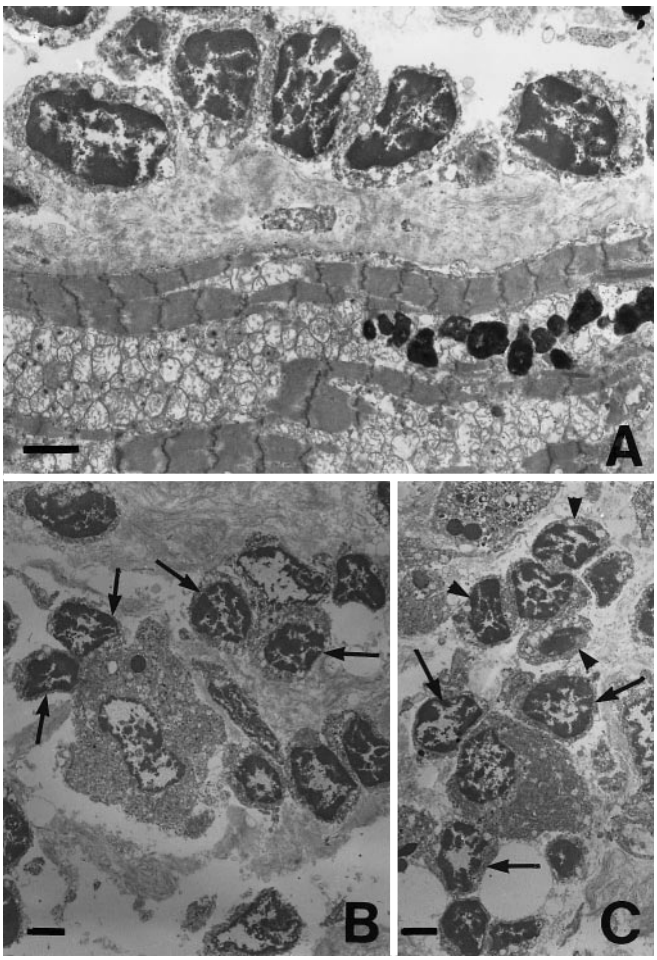

Fig. 3 Electron micrographs of the specimens obtained from the autopsied heart, demonstrating **A** adhesion of many lymphocytes on the cardiac myocytes and **B**, **C** those in the clusters in close contact with macrophages (*arrows*) and another lymphocytes (*arrowheads*). **A** *Bar* 2 µm, original. magnfication ×4000. **B**, **C** *Bar* 2 µm, original magnification ×2400

Expression of cell surface molecules on the cardiac tissue was also investigated (Fig. 4). ICAM-1 and MHC class 1 antigens were expressed in the capillary endothelial cells and on the surface of the cardiac myocytes. VCAM-1 and E-selectin were not expressed in the cardiac tissue.

Discussion

This case showed a gradual onset of cardiac symptoms with clinically progressive deterioration. Histological confirmation of active myocarditis according to the Dallas classification [1] was achieved at biopsy and autopsy, satisfying the diagnostic criteria for chronic active myocarditis proposed by Lieberman et al. [10]. In their study, only 3 of the 348 patients for whom endomyocardail biopsies were performed had a histological diagnosis of

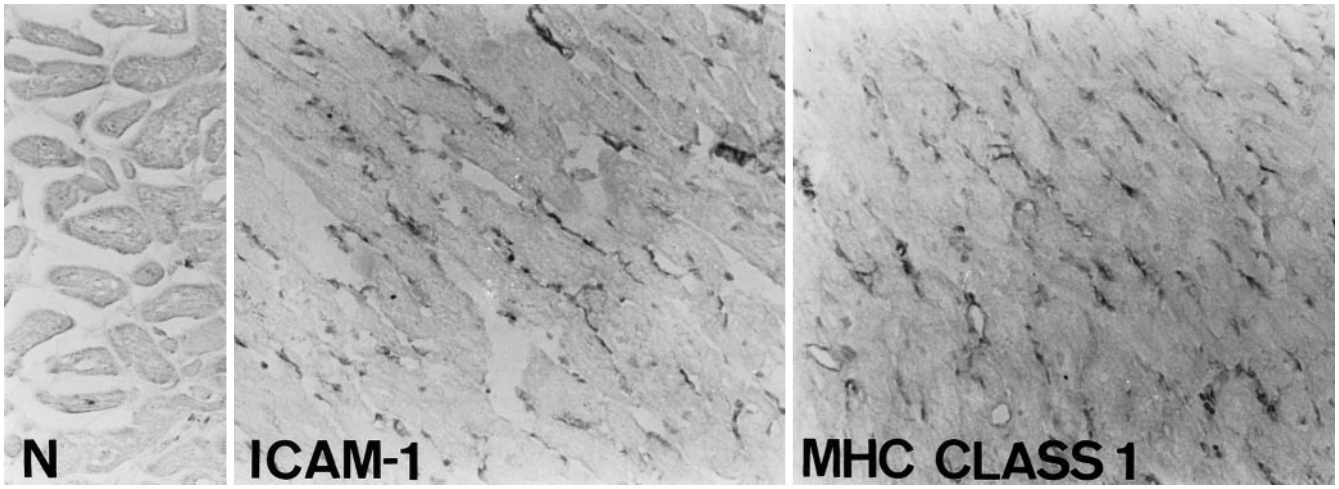

Fig. 4 Expression of cell surface molecules in the autopsied heart. Enhanced expression of ICAM-1 and MHC class 1 antigens are observed in the capillary endothelial cells and on the surface of the cardiac myocytes (*N* negative control)

chronic active myocarditis, and these patients progressed to end-stage dilated cardiomyopathy. According to a Japanese survey [9], this type of myocarditis is rare and a histological diagnosis during the patients life is difficult. However, in our case, which presented as dilated cardiomyopathy, active myocarditis was confirmed by both scintigraphy and histopathological methods.

Another important histological feature, the existence of numerous lymphocytic clusters, was recognized in this case. Post-mortem examinations performed by Okabe et al. [16] for the chronic variant of myocarditis also demonstrated the same pathological findings. However, since immunohistochemical and ultrastructural examination of the lymphocytic clusters was not performed, characterization of the infiltrates remains unclear. In our case, most of the infiltrating cells in the clusters were immunohistochemically positive for CD8, HAM 56 or MHC class 2 antigens, and electron microscopic examinations revealed many lymphocytes in close contact with other infiltrating cells.

Most previous studies on viral acute myocarditis using murine models [5, 7,13, 17, 18] have analysed the cell surface molecules and the characteristics of the infiltrating cells and shown that cell-mediated autoimmunity plays an important part in the process of myocyte injury, nonetheless, its precise role in human chronic myocarditis is poorly understood. It has been documented that the infiltrating cells in the earliest phase consist mainly on natural killer-like large granular lymphocytes and that cytotoxic T lymphocytes become evident in the next phase [17]. One of the cytolytic proteins, perforin, was also found in murine myocarditis. In our case, immunohistochemical analysis revealed a large number of CD8-positive T-lymphocytes around the injured cardiac myocytes. The expression of perforin in the infiltrating cells was also seen around the clusters and in the interstium. Perforin may be related to myocardial cell damage in this disease.

The interaction between killer cells and target cells is mediated by cell surface adhesion molecules [2, 4, 8, 11, 16, 17]. In this case, E-selectin and VCAM-1 were not expressed in cardiac tissue. Although this patient received aggressive corticosteroid therapy, enhanced expression of ICAM-1 and MHC class 1 antigen was observed in the capillary endothelial cells and on the surface of cardiac myocytes. The relationship between corticosteroid treatment and ICAM-1 expression has been examined in extracardiac tissue [3, 6, 14]. In some studies, none of the corticosteroids tested had a significant influence on cytokine-induced ICAM-1 expression [6, 14]. However, in one study corticosteroid therapy apparently decreased ICAM-1 expression [3]. This agrees with the first hypothesis. These findings suggest that, in spite of immunosuppressive therapy, persistent expression of MHC class 1 antigen and of ICAM-1 causes ongoing myocardial inflammation.

Recently a prospective and randomized trial of immunosuppressive therapy in myocarditis was performed [12]; the results of this megatrial have demonstrated that immunosuppressive therapy is not effective. When ICAM-1 is expressed in cardiac tissue, corticosteroids may not be good therapy.

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