Late, persistent expressions of ICAM-1 and VCAM-1 on myocardial tissue in children with lymphocytic myocarditis

Toshihiro Ino a,*, Masahiko Kishiro a, Mataichi Okubo a, Katsumi Akimoto a, Kei Nishimoto a, Keijiro Yabuta a, Ryozo Okada b

a Department of Pediatrics, Juntendo University School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113, Japan
b Cardiovascular Pathology Laboratory, Juntendo University School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113, Japan

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Abstract

Background: Both intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) have been implicated in cardiac allograft rejection. However, there is little information about the relationship between the expression of these adhesion molecules and myocarditis in children.

Methods and Results: Immunoreactivities of ICAM-1 and VCAM-1 were examined by enzyme immunoassay in 31 biopsy specimens obtained from 11 pediatric patients with biopsy-proven myocarditis or cardiomyopathy. Five of the 11 patients had clear evidence of acute myocarditis. The other 6 had ECG abnormalities identified by mass screening for heart disease, and subsequently had been histologically diagnosed as having non-specific cardiomyopathy. The period between onset of myocarditis or identification of ECG abnormality and immunohistochemical studies was 23 to 60 days and 8 months to 3 years, respectively. Expression of ICAM-1 and VCAM-1 was assessed by counting ICAM-1- and VCAM-1-positive vessels and dividing by the total number of vessels. ICAM-1 was significantly present on 81% (P < 0.01) of myocardial tissue samples in the 5 patients with healing-stage acute myocarditis, and on 45% (P < 0.05) in the remaining 6 patients with non-specific cardiomyopathy, compared with 24% in control specimens obtained from right ventricular muscle resected at surgery for tetralogy of Fallot. VCAM-1 was also present on 50% (P < 0.05) of the samples from the 5 patients with acute myocarditis, but was not present in those with non-specific cardiomyopathy. Conclusion: This persistent expression of ICAM-1 suggests that myocardial cell damage may persist immunologically for a long period in myocarditis. In addition, immunostaining for these adhesion molecules may be of diagnostic value in clinically silent lymphocytic myocarditis and chronic cardiomyopathy.

Keywords: Myocarditis; ICAM-1; VCAM-1; Cardiomyopathy; Human

1. Introduction

Cell adhesion molecules are glycoproteins expressed on cell surfaces that allow cell-to-cell contact. Both intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) are important in various immunological processes early in inflammation. ICAM-1 is a ligand for lymphocyte function-associated antigen-1 (LFA-1) and Mac-1, and VCAM-1 is a ligand for α4β1, respectively [1-5]. These are found on lymphocytes, monocytes and polymorphonuclear leucocytes.

Viral myocarditis is usually an acute illness both in children and adults, but may take a subacute or chronic course leading to persistent heart dysfunction or arrhythmia. Recently, the expression of ICAM-1 has been demonstrated on cardiac myocytes both in adult humans with unexplained cardiac dysfunction [6] and animals with acute and healing-stage myocarditis [7,8]. These findings support the hypothesis that in dilated cardiomyopathy the immunological abnormalities persist chronically and may cause chronic inflammation of the myocardium. To confirm this hypothesis, further evaluation in vivo is required, although there is very little information about the expression of ICAM-1 in myocardial disease.

In this study we evaluated the expressions of ICAM-1 and VCAM-1 on cardiac tissues from pediatric patients with biopsy-proven myocarditis or cardiomyopathy.

* Corresponding author. Fax +81 3 5800-0216.

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2. Methods

2.1. Patients

Eleven consecutive patients who were suspected clinically of having myocardial disease underwent right ventricular endomyocardial biopsy between January 1994 and September 1995. The patients' profiles are shown in Table 1. The patients ranged in age from 2 to 16 years (mean 12 years), and the male to female ratio was 8:3. Symptomatic heart failure was present in 4 patients, chest discomfort in 1, and the remaining 6 patients were asymptomatic. These 11 patients were divided into 2 groups according to the mode of identification and clinical manifestation. In the former 5 patients, acute myocarditis was clinically suspected (group 1). All of the latter 6 had abnormal ECGs at initial presentation, which were identified by mass screening for heart disease available in Japan (group 2).

Group 1: In all 5 patients, tachycardia with galloping rhythm and muffled heart sound were initially noted and chest X-ray showed cardiomegaly with cardiothoracic ratio of more than 55%. One patient (No. 4) had ventricular tachycardia with heart rate of 150 beats/min as initial presentation. Two patients with symptomatic heart failure and one (No. 3) with chest discomfort showed significant elevation of the MB isoform of creatinine phosphokinase and a positive CRP value. Viral titers of EB virus and measles were significantly elevated in patients 2 and 3, and a positive CRP value. Viral titers of EB virus and one No. 3 with chest discomfort showed significant elevation of the MB isoform of creatinine phosphokinase and one No. 3 with chest discomfort showed significant elevation of virus titers including Cox-tieviruses and enterovirus. As a result, acute non-specific myocarditis was clinically suspected in the remaining symptomatic 3 patients. Two-dimensional echocardiography performed during the 24 hours before myocardial biopsy revealed reduced left ventricular function with less than 20% fractional shortening in a patient (No. 1). In the acute stage of myocarditis, however, two-dimensional echocardiography showed reduced left ventricular contractility in 4 of the 5 patients. The values of fractional shortening ranged from 9 to 18% in the acute phase of congestive heart failure. During the clinical course, left ventricular function had recovered except in one patient (No. 1). In another patient (No. 4), sustained ventricular tachycardia was noted, but left ventricular fractional shortening was within the normal range during sinus rhythm.

Group 2: Two patients had ventricular tachycardia, 2 had abnormal Q-wave and the remaining 2 had ST-T wave changes at initial presentation of mass screening. Therefore, all of the 6 patients had no symptoms and unknown onsets. Two-dimensional echocardiography showed reduced left ventricular function in only one patient (No. 6).

Four other patients with tetralogy of Fallot served as controls; 11 cardiac muscle specimens were obtained from the right ventricular outflow tract at the time of surgery.

2.2. Light microscopy and pathological criteria of myocarditis

Preparation of specimens: Right ventricular endomyocardial biopsy was performed in all 11 patients after informed consent had been obtained from the parents. Three or 4 biopsy specimens were obtained and each was divided into 2 portions. One portion was fixed in 10% buffered formalin and embedded in paraffin. The other portion was embedded in OCT compound, snap-frozen in

<table>
<thead>
<tr>
<th>Case</th>
<th>Age/yr/sex</th>
<th>Initial presentation</th>
<th>CHF</th>
<th>VT</th>
<th>Duration</th>
<th>%FS</th>
<th>%EF</th>
<th>ICAM-1</th>
<th>VCAM-1</th>
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<tbody>
<tr>
<td>Group 1</td>
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<td></td>
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<tr>
<td>1</td>
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<td>+</td>
<td>–</td>
<td>40 days</td>
<td>19</td>
<td>39</td>
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<td>–</td>
<td>23 days</td>
<td>41</td>
<td>72</td>
<td>+</td>
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<td>3</td>
<td>2 yr/M</td>
<td>fever, VT</td>
<td>+</td>
<td>–</td>
<td>32 days</td>
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<td>70</td>
<td>+</td>
<td>+</td>
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<td>4</td>
<td>11 yr/M</td>
<td>fever, VT</td>
<td>–</td>
<td>+</td>
<td>60 days</td>
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<td>64</td>
<td>+</td>
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<td>–</td>
<td>25 days</td>
<td>30</td>
<td>60</td>
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<td>6</td>
<td>13 yr/M</td>
<td>VT</td>
<td>–</td>
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<td>8 mth</td>
<td>17</td>
<td>36</td>
<td>+</td>
<td>–</td>
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<tr>
<td>7</td>
<td>13 yr/M</td>
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<td>–</td>
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<td>3 yr</td>
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<td>68</td>
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<td>±</td>
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<td>8</td>
<td>16 yr/M</td>
<td>abnormal Q</td>
<td>–</td>
<td>–</td>
<td>1 yr</td>
<td>30</td>
<td>58</td>
<td>±</td>
<td>–</td>
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<tr>
<td>9</td>
<td>12 yr/M</td>
<td>VT</td>
<td>–</td>
<td>+</td>
<td>1 yr</td>
<td>35</td>
<td>63</td>
<td>+</td>
<td>±</td>
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<tr>
<td>10</td>
<td>16 yr/F</td>
<td>abnormal Q</td>
<td>–</td>
<td>–</td>
<td>2 yr</td>
<td>32</td>
<td>62</td>
<td>+</td>
<td>–</td>
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<tr>
<td>11</td>
<td>8 yr/M</td>
<td>ST change</td>
<td>–</td>
<td>–</td>
<td>1 yr</td>
<td>35</td>
<td>65</td>
<td>+</td>
<td>–</td>
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<td>11 ± 5</td>
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CHF = congestive heart failure; EF = ejection fraction; FS = fractional shortening; ICAM-1 = intercellular adhesion molecule-1; VCAM-1 = vascular cell adhesion molecule-1; VT = ventricular tachycardia.

The intensity of staining of ICAM-1 and VCAM-1 in the above patients was compared with that in patients with tetralogy of Fallot. The values for %FS and %EF were obtained on admission for catheterization and biopsy.
Fig. 1. Panels A and B: Photomicrographs of azan staining and immunoreactivity of intercellular adhesion molecule-1 in the healing stage of acute myocarditis (patient No. 1). (A) Biopsy specimen shows focal necrosis, moderate interstitial fibrosis and edema. A moderate disarray of myofibrils can be observed and mononuclear cell infiltration is mild. ×40. (B) ICAM-1 is expressed on interstitial cells and vascular endothelial cells which was identified by hematoxylin-eosin stain. ×100. Panels C and D: Photomicrographs of hematoxylin-eosin staining and immunoreactivity of intercellular adhesion molecule-1 in non-specific cardiomyopathy (patient No. 9). (C) Biopsy specimen shows moderate interstitial fibrosis with sparse mononuclear cell infiltrations. ×100. (D) ICAM-1 is mildly expressed on interstitial cells and vascular endothelial cells (arrow) identified by hematoxylin-eosin staining. ×100.
liquid nitrogen, and stored at −80°C until sectioned. Light-microscopic examination was performed after hematoxylin–eosin, azan and elastica–van Gieson staining.

Criteria of myocarditis: A pathological diagnosis of myocarditis was made according to the Dallas criteria [9] and/or the criteria of the Japan Cardiomyopathy Research Committee [10,11]. The endomyocardial biopsy criteria of the Japanese Circulation Society included acute, subacute, chronic and inactive myocarditis. Acute: large amount of accumulation of large and/or small cells in the myocardium associated with myocyteosis or necrosis of adjacent myocytes, interstitial edema. Subacute: moderate amount of cell infiltration, degeneration, disruption and/or arrangement of myocardial fiber, interstitial fibrosis. Chronic: cell infiltration with myocyteosis, diffuse interstitial fibrosis, irregular replacement of myocardial fibrosis, and fatty degeneration. Hypertrophy, variation in size and irregular arrangement of myocytes. Inactive: similar findings to those in chronic myocarditis but with smaller amount of cell infiltration.

2.3. Immunohistochemistry

In total, 31 specimens were used for immunohistochemical evaluation. Immunohistochemical staining for ICAM-1 and VCAM-1 was performed by the enzyme immunoassay method using mouse monoclonal antibodies against human ICAM-1/CD54 (Cosmo Bio Co., Tokyo, Japan) and human VCAM-1 (R and D Systems Co., Belgium). The sections were cut from the frozen specimens and fixed for 5 min in cold acetone. After washing 5 times with PBS solution for 5 min each, the samples were blocked with 5% horse serum for 20 min at room temperature. After being washed 5 times with PBS solution for 5 min, the samples were reacted with HRPO-labeled goat (anti-mouse IgGα + L) IgGF(ab′)2 (Tago Co., Tokyo, Japan) for 1 h at 37°C. After washing with PBS, the samples were incubated with diaminobenzidine for 5 min at room temperature, and counterstained with hematoxylin for 3 min. Control slides omitted the anti-ICAM-1.

2.4. Evaluation of staining

To allow quantitative assessment of ICAM-1 and VCAM-1 expression, specimens were graded according to the percentage of vessels expressing a particular ICAM-1 and VCAM-1, as reported previously [1,12], using the formula: (number of vessels stained for ICAM-1 or VCAM-1)/[total number of vessels] (×100) (%). The total number of vessels was determined after hematoxylin–eosin staining of specimens obtained from the same block.

A semiquantitative scoring system for ICAM-1 and VCAM-1 staining was also used to evaluate the degree of staining on the other parts of the tissue such as myocyte, interstitial cell and fibroblast as follows: (−) absence of staining, (+) faint staining, (++) moderate staining, (+++) intense staining of cardiac tissue. The individual degree of staining was also represented by a numerical value as follows: (−) = 0, (+) = 1, (++) = 2, (+++) = 3 points. The total summed score was calculated to compare the degree of staining among control, acute myocarditis and non-specific cardiomyopathy. In all biopsies, light-microscopic examination was evaluated blindly by 2 pathologists, and semiquantitative analysis of immunohistochemical staining was independently judged by 2 pediatric cardiologists before comparing the results with clinical findings.

2.5. Statistical methods

Numeric values were represented as mean ± standard deviation. Student’s t-test was used to compare the percentage and score of ICAM-1 and VCAM-1 expressions between groups 1 and 2. A P < 0.05 was defined as statistically significant.

3. Results

3.1. Results of light microscopy

The subjects studied here were clinically and histologically divided into 2 groups according to the light-microscopic findings: Group 1, 5 patients with unequivocal lymphocytic myocarditis; Group 2, 6 patients with non-specific cardiomyopathy. Five patients (Nos. 1–5) of group 1 in whom clinical manifestations of acute myocarditis including onset were evident had histological findings of healing-stage acute myocarditis. These findings included focal necrosis and disarray of myofibers, mild but significant mononuclear cell infiltration and interstitial fibrosis. The mononuclear cell infiltration was present in perivascular and interstitial areas. Some of the cells were partly attached to myocytes. The remaining 6 patients (Nos. 6–11) of group 2 had mild non-specific cardiomyopathy with moderate interstitial fibrosis, disarray of myofibers and sparse mononuclear cell infiltration. In these patients, interstitial fibrosis and myofiber disarray were rather more prominent histological features than cell infiltration.

3.2. Results of immunohistochemistry

The degrees of ICAM-1 and VCAM-1 staining in all specimens are summarized in Table 2. Specimens with

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<tr>
<td>Quantitative analysis of ICAM-1 and VCAM-1 expression in myocarditis and cardiomyopathy</td>
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<tr>
<td>ICAM-1</td>
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<tr>
<td>Specimens</td>
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<tr>
<td>Control (n = 4)</td>
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<tr>
<td>Acute myocarditis (n = 5)</td>
</tr>
<tr>
<td>Cardiomyopathy (n = 6)</td>
</tr>
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* P < 0.01 vs control. ** P < 0.05 vs control. * P < 0.05 vs **.
Table 3
Semi-quantitative scoring of ICAM-1 and VCAM-1 expression in myocarditis and cardiomyopathy

<table>
<thead>
<tr>
<th></th>
<th>ICAM-1</th>
<th>VCAM-1</th>
<th>n</th>
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<tbody>
<tr>
<td>Control</td>
<td>0.5 ± 0.6 *</td>
<td>0.25 ± 0.5 *</td>
<td>4</td>
</tr>
<tr>
<td>Acute myocarditis</td>
<td>2.8 ± 0.4 **</td>
<td>2.0 ± 0.5 *</td>
<td>5</td>
</tr>
<tr>
<td>Cardiomyopathy</td>
<td>1.8 ± 0.4 ***</td>
<td>0.3 ± 0.5</td>
<td>6</td>
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P < 0.005 in * vs **. P < 0.003 in * vs ***. P < 0.001 in b vs a.

There was no significant difference between groups 1 and 2, and between group 2 and the control. In quantitative analyses, there was no clear, significant correlation between the degree of staining and clinical parameters such as left ventricular function, the level of cardiac enzymes or duration of fever. In 4 patients with heart failure, however, intense staining of ICAM-1 was more evident than in the other 7 patients.

The results of semi-quantitative scoring for ICAM-1 and VCAM-1 were identical between 2 independent pediatric cardiologists. As shown in Tables 1 and 3, intense ICAM-1 immunoreactivity was observed on cardiac myocytes, interstitial cells and fibroblasts as well as vascular endothelial cells in 5 patients with acute myocarditis (Fig. 1A,B). In the remaining 6 patients, ICAM-1 was also expressed mildly to moderately on myocytes and endothelial cells (Fig. 1C,D). Similarly, VCAM-1 was mildly expressed on vascular endothelial cells in patients with acute myocarditis. There was no significant expression of VCAM-1 on cardiac tissue in the 6 with non-specific cardiomyopathy. In the 4 patients with tetralogy of Fallot used as controls, ICAM-1/VCAM-1 were faintly expressed only on the vascular endothelium in 6 and 5 of the 11 specimens, respectively.

4. Discussion

4.1. Histological diagnosis of chronic myocarditis

In the present study, non-specific cardiomyopathy was eventually diagnosed by histological examination in 6 patients of group 2 without clear clinical features of myocarditis according to the Dallas criteria [9]. The most common histological features in these patients included moderate interstitial fibrosis and sparse mononuclear cell infiltration with/without mild myofibril disarray and hypertrophy. The number of infiltrated mononuclear cells per field was less than 5 at high magnification. These features were similar to those seen in biopsy samples from patients with chronic, inactive myocarditis or idiopathic dilated cardiomyopathy. However, the clinical features including ventricular function determined by echocardiographic evaluation were not consistent with those of dilated cardiomyopathy. Mason and colleagues recently reported a clinical trial of immunosuppressive therapy for myocarditis [13]. In their study, only 214 (10%) of 2233 patients with clinically suspected myocarditis had positive pathology of myocarditis consistent with Dallas criteria. This is very surprising and raise the question of whether the Dallas criteria is too rigorous for diagnosis, as McKenna and colleagues stated in their editorial comments [14]. We believe that these histological findings may have resulted from chronic inflammation, being consistent with those in chronic or inactive myocarditis, although repeat biopsy was not performed in these pediatric patients. These findings were satisfied by the criteria of chronic or inactive myocarditis from the Japan Cardiomyopathy Research Committee [10,11].

4.2. Significance of ICAM-1 expression in myocarditis

The immunological mechanism of myocyte damage in viral myocarditis has recently been clarified by experimental studies of Coxsackie B3 virus myocarditis [7,8]. Cell adhesion molecules are considered to play an important role in cell–cell interactions between myocytes and immune cells. Toyozaki and colleagues [6] have reported that intense expression of ICAM-1 on myocardial tissue was detected in some patients with unexplained ventricular dysfunction, being clinically compatible with dilated cardiomyopathy. This strongly suggests that the myocardial cell damage involved in viral myocarditis may persist for a long period and may eventually lead to dilated cardiomyopathy. In animal studies of acute myocarditis, the expression of ICAM-1 on myocytes was found to persist for only 4 weeks after virus inoculation [7]. In our study, the expression of both ICAM-1 and VCAM-1 was still evident in 5 patients with acute myocarditis even though right ventricular biopsy had been performed 20–60 days after onset. The intensity of staining was increased to a greater extent in ICAM-1 than in VCAM-1. The duration between onset of myocarditis and biopsy was much longer in the patients with acute myocarditis in this study than in animals with acute myocarditis. Thus, the present findings seem to differ from those of experimental studies in terms of the relationship between the expression of ICAM-1 and the duration from onset.

In non-specific cardiomyopathy of unknown onset, only mild ICAM-1 expression was observed without VCAM-1 expression. This may indicate the persistence of chronic inflammation in this type of cardiomyopathy. In addition, the persistent expression of ICAM-1 even in clinically silent cardiomyopathy suggests that myocardial cell damage due to chronic inflammation may persist for a long period and may contribute to the abnormal ECG in af-


dermann pathology consistent with chronic, inactive myocarditis or idiopathic dilated cardiomyopathy. However, the clinical features including ventricular function determined by echocardiographic evaluation were not consistent with those of dilated cardiomyopathy. Mason and colleagues recently reported a clinical trial of immunosuppressive therapy for myocarditis [13]. In their study, only 214 (10%) of 2233 patients with clinically suspected myocarditis had positive pathology of myocarditis consistent with Dallas criteria. This is very surprising and raise the question of whether the Dallas criteria is too rigorous for diagnosis, as McKenna and colleagues stated in their editorial comments [14]. We believe that these histological findings may have resulted from chronic inflammation, being consistent with those in chronic or inactive myocarditis, although repeat biopsy was not performed in these pediatric patients. These findings were satisfied by the criterion of chronic or inactive myocarditis from the Japan Cardiomyopathy Research Committee [10,11].

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fected patients. In contrast, such abnormal ECGs, identified by mass-screening for heart disease, may be the result of chronic inflammation.

4.3. Difference between ICAM-1 and VCAM-1 expression

Previous studies have demonstrated that VCAM-1 is expressed not on capillary vessels but rather on large venules, which are not always present in tiny biopsy specimens [15,16]. Although little information regarding the relationship between VCAM-1 expression and myocarditis has been obtained, there was mild but significant expression of VCAM-1 at the healing stage of acute myocarditis in our study. In these patients, the expression of VCAM-1 was less intense and less persistent than that of ICAM-1. These results suggest that the intensity of ICAM-1 staining and the presence or absence of VCAM-1 in myocardial tissue may be of diagnostic significance in the healing stage of acute myocarditis or chronic myocarditis. VCAM-1 expression seems to be less important than ICAM-1 expression in myocarditis in this setting. Therefore, both ICAM-1 and VCAM-1 expression on myocardial tissue strongly indicates the presence of acute to subacute inflammation, and the presence of only ICAM-1 expression alone may not support acute inflammation.

4.4. Limitations of the study

The present study used a quantitative approach for determination of ICAM-1 and VCAM-1 expression. This method was used after referring to descriptions of previous reports. Tanio and colleagues used PECAM-1 staining to identify the total number of vessels in the tissue [1]. In the present study, vessel identification was performed after hematoxylin-eosin staining and not PECAM-1 staining. There may be some limitation in counting the number of small capillary vessels accurately. However, it was possible to compare the percentages of ICAM-1- and VCAM-1-positive cells between myocarditis and controls. Specific marker of endothelial cell surfaces such as Von Willebrand factor or PECAM-1 may be more accurate in identifying capillary vessels.

Specimens obtained from patients with tetralogy of Fallot may be inappropriate as normal controls because the myocytes are always hypertrophic due to the ventricular pressure load. On light microscopy, however, there was no evidence of myocyte necrosis or significant cell infiltration around the myocytes. These findings indicated no inflammatory process in the myocardial tissue, and therefore it was considered possible to use these tissues from tetralogy of Fallot cases as controls.

5. Conclusions

In summary, our study supports a relationship between myocarditis and ICAM-1 expression. ICAM-1 expression was intense in acute lymphocytic myocarditis but mild in non-specific cardiomyopathy without obvious onset, perhaps indicating the persistence of inflammation. The expression of VCAM-1 may be less important than that of ICAM-1. In addition, staining for these adhesion molecules may be of diagnostic value in clinically silent lymphocytic myocarditis.

Acknowledgements

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References