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Induction of Major Histocompatibility Complex Antigens Within the Myocardium of Patients With Active Myocarditis: A Nonhistologic Marker of Myocarditis

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The histologic diagnosis of active myocarditis is frequently difficult to establish. A nonhistologic marker of immune activation would be clinically useful in identifying cases of immune-mediated myocarditis. A viral etiology with subsequent autoimmunity to cardiac antigens has been implicated in human myocarditis. Because autoimmunity and viral disease are commonly associated with increased expression of major histocompatibility complex (MHC) antigens on targeted tissue, we examined endomyocardial biopsy samples from patients with active myocarditis for abnormal levels of MHC antigen expression. Thirteen patients with active myocarditis and eight control patients with other well-defined cardiac diagnoses (coronary disease, amyloidosis or neoplasm) were studied.

A sensitive radioimmunoassay was developed that utilized monoclonal antibodies to human MHC class I and class II antigens in order to quantitate the expression of both of these antigens within each biopsy. Abnormal MHC class I and class II antigen expression was present in 11 of 13 myocarditis specimens and 1 of 8 control samples (specificity 88%, sensitivity 84.6%). Active myocarditis samples had approximately a 10-fold increase in MHC class I and class II expression. Immunoperoxidase staining localized abnormal MHC expression primarily within microvascular endothelium and along myocyte surfaces (11 of 13).

This study is the first to demonstrate a marked increase in major histocompatibility complex antigen expression within the myocardium of patients with active myocarditis. The identification of abnormal histocompatibility antigen expression within an endomyocardial biopsy may prove a useful adjunct to the histologic diagnosis of myocarditis.

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Experience with the role of endomyocardial biopsy in patients with suspected myocarditis has resulted in the development of a reproducible histologic definition of active myocarditis (1). The finding of both myocardial inflammation and myocyte necrosis establishes the diagnosis of active myocarditis. Although the diagnosis can be made reproducibly by expert cardiac pathologists, certain histologic features of human myocarditis may make the diagnosis difficult to establish in all cases. The inflammatory cell infiltrates are frequently focal and, in all but the most severe cases of myocarditis, foci of myocyte necrosis may be difficult to locate. Nonhistologic markers within the myocardium that would be uniformly expressed throughout the tissue sample would be clinically useful as an adjunct to the histologic diagnosis.

Chagasic (2) and rheumatic myocarditis (3) are both clearly associated with infectious agents, and recent identification of enteroviral RNA within endomyocardial biopsy samples of idiopathic myocarditis supports the hypothesis that many cases of myocarditis may be initiated by a viral infection (4). This hypothesis has broad support in both clinical and experimental studies (5–16) and, although the precise role of virus or viruses in myocarditis is not yet fully

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defined, postviral autoimmunity to cardiac-specific antigens has been described in both animal models and human myocarditis.

Major histocompatibility complex (MHC) molecules play an important role in presenting antigens such as a virus to the immune system. T lymphocytes thus recognize these antigens only in the context of self-MHC molecules, a phenomenon known as MHC-restriction (17,18). MHC class I molecules are present to some extent on virtually all nucleated cells (18,19) and, in association with viral antigens, appear to be the target of sensitized, predominantly $CD8^+$ cytotoxic T cells. MHC class II molecules, on the other hand, are expressed primarily on antigen presenting cells of the immune system such as macrophages and B cells and in the presence of processed antigen predominantly activated $CD4^+$ (helper) T cells (20). Increased expression of these MHC antigens has been demonstrated in human tissues undergoing autoimmune injury (20–22), allograft rejection (23–26), viral diseases and other inflammatory states (27–29). Normal human fetal and adult cardiac myocytes express low levels of MHC class I (HLA, A, B and C regions) antigens and do not express detectable levels of MHC class II (HLA DP, DQ and DR regions) antigens (19,20,30,31).

We used monoclonal antibodies directed against monomorphic determinants of human MHC class I and class II antigens to examine endomyocardial biopsy specimens from selected patients with idiopathic myocarditis to determine whether altered expression of MHC antigens could be detected within the myocardium. The control group comprised patients with no histologic evidence of myocarditis on endomyocardial biopsy who were later found to have clear-cut clinical and histopathologic diagnoses explaining their left ventricular dysfunction.

Methods

Study patients. Between June 1986 and April 1987, 80 patients with clinically suspected myocarditis underwent endomyocardial biopsy. All patients had some degree of left ventricular dysfunction after an initial presentation of rapid onset congestive heart failure and inadequate clinical response to conventional therapy with diuretics, digoxin and, frequently, vasodilators. All patients underwent initial evaluation including history, physical examination, routine hemograms and serum chemistry determinations, screening for collagen-vascular disease, thyroid function testing and screening for pheochromocytoma. All patients then gave informed consent and underwent endomyocardial biopsy and right heart catheterization. At the time of catheterization, serum was obtained for identification of heart autoantibodies. Written informed consent for this protocol was also obtained.

Endomyocardial biopsy. Biopsy was performed by way of the right internal jugular vein with a modified Caves bioptome. During the procedure a minimum of three separate endomyocardial biopsy specimens were obtained for routine histologic examination and one specimen was frozen for immunohistochemistry studies.

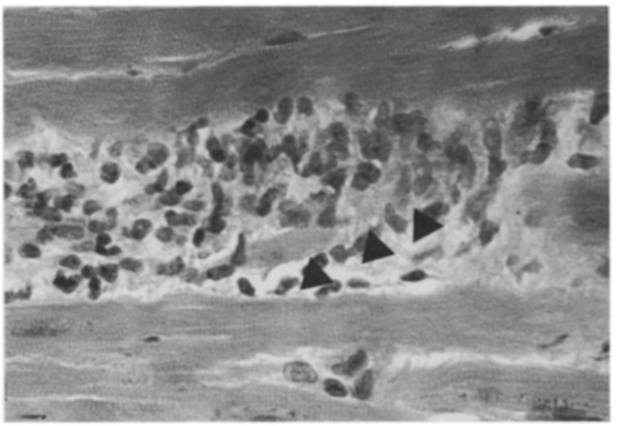

Figure 1. Photomicrograph of an endomyocardial biopsy sample from a patient with active myocarditis, showing the focal interstitial collection of inflammatory cells surrounding a necrotic myocyte (**arrows**) (hematoxylin-eosin stains; ×350).

Histology. All biopsy specimens were serially sectioned and examined with use of hematoxylin-eosin staining. Five serial sections of 5 μm thickness were cut and placed on each of seven glass slides so that the majority of the biopsy tissue was examined. Each set of biopsy specimens was examined by two cardiac pathologists who were unaware of patient data. Myocarditis was defined according to the Dallas criteria as inflammation of the myocardium associated with necrosis and degeneration of adjacent myocytes not typical of the ischemic damage seen with coronary artery disease (1) (Fig. 1).

Semiquantitation of major histocompatibility complex class I and class II expression. Endomyocardial biopsy sections were assessed for relative levels of MHC class I and class II expression using standard immunoperoxidase techniques. Frozen sections were treated with either monoclonal antihuman class I (clone W6/32, ATCC), antihuman class II (clone L-243, ATCC) or normal mouse serum, and incubated with biotin-conjugated rabbit antimouse immunoglobulin (Ig) and an avidin-biotinylated horseradish peroxidase complex (Vector Labs). After washing, sections were incubated with a peroxidase substrate, amino ethylcarbazole (AEC), washed and mounted.

Triplicate slides were coded and the relative reactivity was scored semiquantitatively by two independent observers. With each experimental run, tissues from an autopsy, normal control case and a case of fatal coxsackie B_3 virus culture, positive myocarditis were included. The biopsies were scored for levels of MHC antigen expression on capillary, venous and arteriolar endothelium as well as for MHC expression on myocytes. The final results represent the

consensus of the two individuals who scored the entire panel of slides. *Immunoperoxidase staining was scored as follows*:

0 to 1+ = weak, focal staining similar to that seen in negative control samples; myocytes are negative, endothelial cells lining the vessels usually contributing to the weak staining.

2+ = endothelial cells more positive; myocyte sarcolemmal surfaces are negative.

3+ = endothelial cells lining both venous and arteriolar beds are strongly positive; myocyte surfaces are focally positive, whereas cytoplasmic structures and intercalated discs have a weak, diffuse staining.

4+ = intense diffuse staining of endothelial cells and multifocal myocyte sarcolemmal staining.

Quantitation of MHC class I and class II expression. The radioimmunoassay was performed by removing the OCT compound from each biopsy specimen and cutting 5 μm sections. Four sections were transferred to glass tubes previously coated overnight with 5% bovine serum albumin, and each assay was performed in triplicate. Each tube contained 3 ml of medium (RPMI 1640 supplemented with 100 μg/ml streptomycin, 100 U/ml penicillin, 2 m*M* L-glutamine and 5% heat-inactivated fetal calf serum) containing 0.1 *M* phenyl methyl sulphonyl fluoride (Sigma Chemical). After incubation at 4°C for 20 min, tubes were centrifuged at 650 *g*, the fluid was drained and 3 ml of RPMI medium containing 5% normal mouse serum was added. After incubation at 4°C for 20 min, to each set of triplicate tubes was added either normal mouse serum (control), antihuman cardiac myosin (EU-1H1), antihuman monomorphic MHC class I (W6/32) or antihuman monomorphic MHC class II (L-243). The tubes were vortexed, incubated for 60 min at 4°C and washed twice with phosphate-buffered saline solution pH 7.4. To each tube, RPMI medium containing 5% normal goat serum was added (3 ml/tube) and following incubation at 4°C for 20 min the fluid was drained and affinity purified Iodine-125–labeled goat antimouse immunoglobulin (100 μl, 1:50 containing 50,000 counts/min) was added. After gentle mixing and incubation for 30 min at 4°C, the tubes were washed three times with phosphate-buffered saline solution and then counted in an LKB gamma counter. Autopsy tissue sections from a normal human heart (from a case involving trauma) and tissue sections from the heart of a patient who died of acute myocarditis were run in parallel and served as negative and positive controls, respectively. Mean values ± SD in counts/min for the triplicate samples were calculated. Any assay giving less than a fivefold increase in activity between the negative and positive control was rejected and, hence, repeated.

To adjust for differences in the size of each biopsy sample used in the radioimmunoassay, a monoclonal anticardiac myosin reagent was used. Cardiac myosin is the major intracellular protein within the myocardium and, therefore, reflects the quantity of tissue examined in the radioimmunoassay. Data are expressed as raw counts/min or as the ratio of class I or class II activity to myosin activity.

Indirect immunofluorescence. Standard techniques for indirect immunofluorescence were used (32). Heart, kidney, liver, skeletal muscle and stomach were removed from 6 month old Sprague-Dawley rats (Charles River) and frozen and 4 μm sections were cut in a cryotome. The sections were overlaid with human serum diluted 1:10, 1:20, 1:40 and 1:80 and incubated with goat antihuman Immunoglobulin G, crystallization fraction (Fc) gamma chain specific (Organon 1202-0121, lot # 27107), 50 μl FITC at 1:40 dilution. A rhodamine counterstain was used at 20 μl/ml (Difco Laboratories). The slides were rinsed and washed for 30 min in phosphate-buffered saline solution. The sections were mounted with 90% (vol/vol) glycerol/phosphate-buffered saline solution and examined using a Zeiss fluorescent microscope.

Statistical analysis. Comparisons between the two patient groups were made with use of the Student's *t* test. Proportions were analyzed using the chi-square test. Linear and logistic regression analyses were used to determine the relations among clinical, serologic and immunohistochemical data. Differences achieved statistical significance when two-tailed p values were <0.05. Group means are expressed as mean values ± SD, unless otherwise noted.

Results

Endomyocardial biopsies. All 80 endomyocardial biopsy specimens contained at least three separate endomyocardial fragments for routine examination by hematoxylin-eosin staining (mean 5.7 fragments). Thirteen (16%) of the 80 patients had biopsy specimens that fulfilled the histologic criteria for active myocarditis (Fig. 1). In addition, 8 patients (10%) had no evidence of myocarditis, but other clear-cut clinical or histopathologic diagnoses were established (discussed later). These eight patients constitute the control group. The remaining 59 patients (73.5%) who are not reported on in this study had either normal appearing biopsy specimens or specimens showing various degrees of myocyte hypertrophy and interstitial fibrosis, consistent with the diagnosis of idiopathic cardiomyopathy or borderline myocarditis.

Clinical data (Table 1). *A description of the patient's age, sex, clinical diagnosis, New York Heart Association functional class and ejection fraction is given in Table 1.* The patients with myocarditis were younger than the control group without myocarditis (38.7 ± 14.2 versus 53.4 ± 15.1 years, p = 0.035). There were no significant differences between the two groups with respect to gender, functional class or ejection fraction. Of the 13 patients with myocarditis, 3 had postpartum myocarditis (Patients 6, 11 and 12), whereas the remaining 10 had idiopathic active myocarditis. Of the 8 control patients, 5 (Patients 14 to 18) were found to have severe coronary disease on subsequent left heart cath-

Table 1. Clinical Data on 13 Patients With and 8 Without Myocarditis. Comparison of Semiquantitative Scores for MHC Antigen Expression in Endomyocardial Biopsy Samples Using Immunoperoxidase

Patient No.	Age (yr)/ Gender	NYHA Class*	Ejection Fraction (%)	Relative Levels of MHC Antigen Expression by Immunoperoxidase†	
				Class I	Class II
Myocarditis Group					
1	25/M	3	22	3	3
2	54/F	3	18	2	4
3	22/F	4	20	3	3
4	27/M	2	36	3	3
5	58/M	3	17	2	3
6	38/F	4	23	4	4
7	27/M	2	27	3	2
8	53/F	3	29	3	1
9	48/M	2	27	3	1
10	28/F	3	30	1	0
11	31/F	2	26	2	3
12	30/F	3	20	1	0
13	62/M	3	23	4	3
Mean	38.7	2.9	24.2	2.6	2.3
±SD	14.2	0.7	5.5	1.0	1.4
Nonmyocarditis Group					
14	67/M	3	20	0	1
15	42/M	3	23	1	0
16	60/M	4	20	0	1
17	49/M	3	19	1	0
18	33/M	2	24	2	0
19	75/M	3	20	1	2
20	38/F	2	45	2	0
21	63/F	2	40	2	0
Mean	53.4	2.8	26.4	1.1	0.5
±SD	15.1	0.7	10	0.8	0.8
p Values	0.035	NS	NS	0.002	0.002

*New York Heart Association functional class. †semiquantitative scoring from 0 to 4. F = female; M = male; MHC = major histocompatibility complex; NS = not signficant.

eterization, 2 (Patients 19 and 21) were found to have significant amyloid infiltration of the myocardium by endomyocardial biopsy and 1 patient (Patient 20) had a significant pericardial effusion secondary to metastatic esophageal carcinoma.

Immunohistochemistry. Normal cardiac tissue from control autopsy samples did not show greater than grade 1 major histocompatibility complex (MHC) class I antigen expression by the immunoperoxidase method. Myocytes appeared uniformly negative; however, within the interstitium there was a faint staining pattern in what appeared to be endothelial cells lining the microcirculation (Fig. 2). MHC class II antigen expression was essentially negative except for rare interstitial cells that were faintly positive. Although the identity of these cells is not known, they likely represent interstitial dendritic cells. Positive autopsy control cases consisted of cases of culture-proved viral myocarditis in which the myocytes and microvascular endothelium appeared uniformly and intensely positive for both MHC class I and MHC class II antigens. Three of the eight patients in the nonmyocarditis control group (Patients 18, 20 and 21) had a grade 2 immunoperoxidase score for MHC class I antigen in consistent with increased expression of endothelial MHC class I antigen (specificity 63%) (Fig. 3). Only one of these eight control patients (Patient 19) had a grade 2 score for MHC class II antigen expression (specificity 88%). None of the nonmyocarditic control samples had myocyte

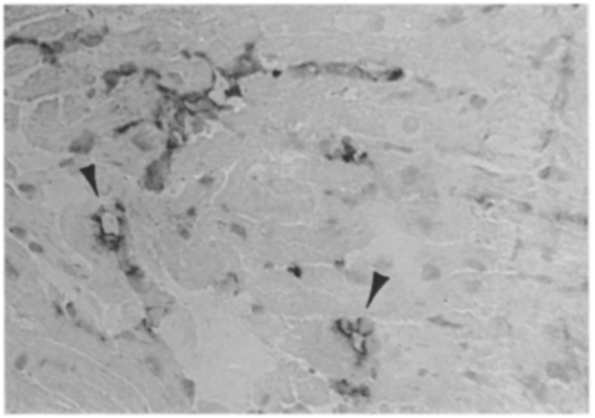

Figure 2. Control autopsy specimen from a patient with no history of heart disease. Frozen section treated with monoclonal antihuman MHC class I antigen using standard immunoperoxidase technique; Faint staining of capillary endothelium scattered throughout the sample (**arrows**) (myocytes do not stain; ×250).

Figure 3. Endomyocardial biopsy frozen section sample from nonmyocarditis control patient with grade 2 immunoperoxidase score for MHC class I antigen expression, with increased staining intensity of the microvascular endothelium compared with Figure 2 (myocytes do not stain; ×160).

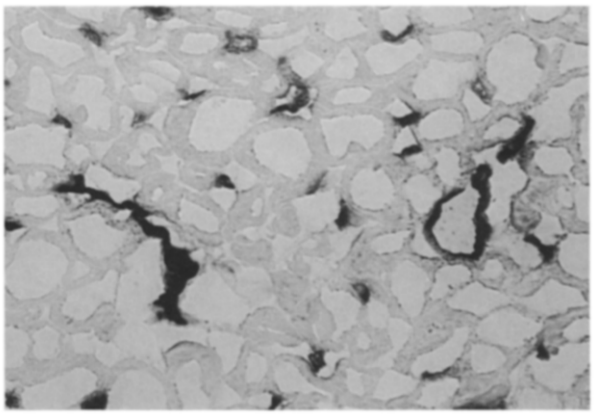

Table 2. Major Histocompatability Complex (MHC) Class I and Class II Antigen Expression as Determined by Radioimmunoassay With the Presence of Circulating Heart Autoantibodies (IFA) in Patients With and Without Myocarditis

Patient No.	Class I*	Class II*	Class I†	Class II†	Autoantibodies
			Myocarditis		
1	21,721	36,587	1.39	2.35	Yes (1:80)
2	18,016	28,057	0.87	1.37	Yes (1:80)
3	11,696	14,751	1.19	1.51	Yes (1:80)
4	15,775	21,981	1.25	1.74	Yes (1:80)
5	1,449	7,050	0.09	0.45	Yes (1:40)
6	25,483	33,671	2.02	2.67	Yes (1:20)
7	13,351	7,168	0.84	0.45	No
8	11,700	6,398	0.69	0.38	No
9	27,254	16,999	1.32	0.83	No
10	1,280	482	0.09	0.03	No
11	8,331	12,298	0.41	0.61	No
12	4,438	1,456	0.19	0.06	Yes (1:20)
13	31,276	25,782	1.60	1.32	Yes (1:80)
Mean	14,752	16,360	0.92	1.06	
±SD	9,710	12,007	0.61	0.85	
			Nonmyocarditis		
14	2,022	680	0.16	0.05	No
15	1,253	2,347	0.07	0.13	No
16	1,231	3,898	0.08	0.24	No
17	631	684	0.04	0.04	No
18	635	171	0.04	0.01	No
19	1,860	2,024	0.10	0.11	No
20	739	1,748	0.03	0.07	NA
21	1,077	757	0.06	0.04	NA
Mean	1,181	1,833	0.070	0.087	
±SD	532	1,222	0.043	0.07	
p values	0.0003	0.003	0.001	0.005	

*raw counts/min. †ratio of class I and class II counts/min to antimyosin counts/min. IFA = indirect immunofluorescene assay; NA = not available.

MHC class I or class II antigen expression (specificity 100%).

The active myocarditis group revealed a significant increase in expression of both MHC class I and class II antigens, by both semiquantitative and quantitative methods (Tables 1 and 2). The cardiac interstitium is frequently a compressed space and precise identification of peroxidase-positive stained interstitial cells would require immunoelectron microscopic technique not employed in this study. The morphologic appearance of the majority of the positive-stained cells is that of microvascular endothelial cells. The presence or absence of MHC antigen expression on Purkinje cells could not be determined with the standard immunoperoxidase techniques used in this study.

Microvascular endothelial and sarcolemmal staining. Increased MHC class I microvascular endothelial staining (≥ grade 2 by immunoperoxidase) was seen in 11 of 13 myocarditis samples (sensitivity 84.6%), whereas MHC class II endothelial staining was increased in 9 of 13 samples (sensitivity 69%). Eight of myocarditis samples had myocyte sarcolemmal staining for MHC class I (≥ grade 3 by immunoperoxidase) (sensitivity 62%). Similarly, 8 of the 13 samples had sarcolemmal staining for MHC class II (sensitivity 62%) (Table 1, Fig. 4). Only two active myocarditis samples did not have increased expression of either MHC class I or MHC class II on myocytes (Patients 10 and 12). Some cases with intense microvascular and sarcolemmal staining had a faint diffuse staining within myocyte cytoplasm. This pattern was considered an artifact, because we are not aware of any precedent for MHC expression within the cytoplasm of cells.

Sensitivity and specificity. Radioimmunoassay results on MHC class I and II expression are expressed as the mean of three separate measurements. Standard deviations for these triplicate measurements did not differ from the raw count by more than 4% to 9%. Raw counts per patient biopsy are listed in Table 2. Data normalized for variations in biopsy size from patient to patient are expressed as a ratio of class I and class II to myosin content (Table 2, Fig. 5). *For the radioimmunoassay, abnormal expression of MHC class I and class II antigens was defined as any value above 2 SD above the mean of values from the nonmyocarditis control group (expressed as ratios of counts/min to cardiac myosin (class I > 0.159, class II > 0.235).* With the use of these ratios in seven of eight control specimens (specificity 88%), values in each fell within the normal range for class I expression as well as class II expression (Table 2). In the myocarditis group, 2 of 13 samples (from Patients 5 and 10) fell within the designated normal range for MHC class I expression (sensitivity 84.6%), whereas 2 of 13 samples (from Patients 10 and 12) fell within the designated normal range for MHC class II expression. These patients could not be histologically or clinically distinguished from the other patients with myocarditis.

Comparison of radioimmunoassay and immunoperoxidase techniques. Linear regression analysis revealed significant correlations between the radioimmunoassay technique both for quantitative MHC class I and class II expression and their respective semiquantitative immunoperoxidase scores (MHC class I: $R = 0.911$, $R^2 = 0.830$, $p < 0.001$; MHC class II: $R = 0.825$, $R^2 = 0.681$, $p < 0.001$). Multiple logistic regression analysis identified both the raw counts/min for MHC class I ($p < 0.0005$) and the ratio of class II to myosin content ($p = 0.008$) as independent and additive predictors of a positive biopsy for active myocarditis. The specificities, sensitivities and predictive values for various combinations of quantitative variables that distinguish myocarditis samples are listed in Table 3. This logistic regression indicates that semiquantitative immunoperoxidase scores are not as significantly predictive of active myocarditis as the quantitative radioimmunoassay measurements.

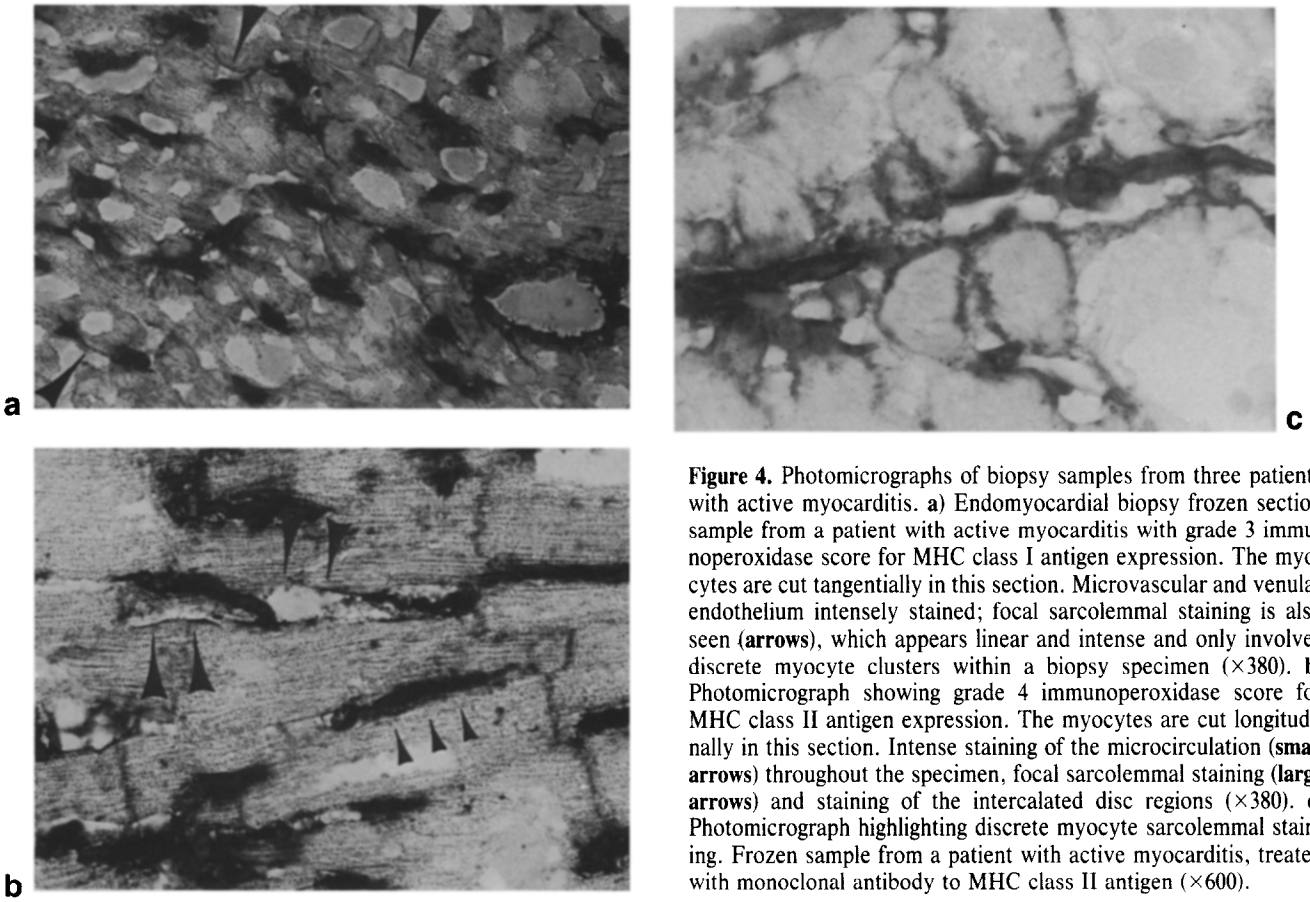

Figure 4. Photomicrographs of biopsy samples from three patients with active myocarditis. **a)** Endomyocardial biopsy frozen section sample from a patient with active myocarditis with grade 3 immunoperoxidase score for MHC class I antigen expression. The myocytes are cut tangentially in this section. Microvascular and venular endothelium intensely stained; focal sarcolemmal staining is also seen (**arrows**), which appears linear and intense and only involves discrete myocyte clusters within a biopsy specimen (×380). **b)** Photomicrograph showing grade 4 immunoperoxidase score for MHC class II antigen expression. The myocytes are cut longitudinally in this section. Intense staining of the microcirculation (**small arrows**) throughout the specimen, focal sarcolemmal staining (**large arrows**) and staining of the intercalated disc regions (×380). **c)** Photomicrograph highlighting discrete myocyte sarcolemmal staining. Frozen sample from a patient with active myocarditis, treated with monoclonal antibody to MHC class II antigen (×600).

Comparison between MHC class I and II expression within each group. Within the myocarditis group, both MHC class I and class II expressions were similarly increased (semiquantitative immunoperoxidase: 2.6 ± 1.0 and 2.3 ± 1.4, respectively; quantitative radioimmunoassay: 0.92 ± 0.61 and 1.06 ± 0.85, respectively). Similarly, no significant differences were present between MHC class I and class II expressions within the nonmyocarditis control group (semiquantitative immunoperoxidase 1.1 ± 0.8 versus 0.5 ± 0.8; quantitative radioimmunoassay, 0.07 ± 0.04 versus 0.09 ± 0.07, respectively).

Circulating autoantibodies. Significant titers of heart autoantibodies (immunoglobulin G isotype) were identified in serum from 8 of 13 patients with myocarditis (prevalence 61.5%) but were not present in any of the 6 control patients where serum was available for study. Immunoglobulin G (IgG) heart autoantibody titers of ≥20 are seen in only 4% of normal volunteer control subjects (unpublished data). Linear regression analysis revealed good correlation between both quantitative MHC class I expression and circulating Immunoglobulin G heart autoantibody titer ($r = 0.593$, $p = 0.006$), and quantitative MHC class II expression and circulating Immunoglobulin G heart autoantibody titer ($r = 0.708$, $p < 0.001$). Of the eight patients with myocarditis with significant titers of circulating heart autoantibodies, seven had abnormal expression of MHC class II antigens by both the immunoperoxidase and the radioimmunoassay methods.

Discussion

This study provides the first evidence that human myocarditis is associated with abnormal myocardial expression of class I and class II major histocompatibility complex (MHC) antigens. The data support the hypothesis that in many cases of active myocarditis, both myocytes and interstitial components such as microvascular endothelium are the specific targets of immune-activated inflammatory cells.

Mechanisms: the role of the major histocompatibility complex in presenting cardiac antigen or antigens. The histologic hallmark of active myocarditis is the presence of inflammatory cells within the myocardium associated with focal myocyte necrosis. A leading hypothesis suggests that many cases of myocarditis are triggered by infection with a cardiotropic virus (5–16), which may be followed by a subacute or chronic immune response within the myocardium. Cardiac antigens could be liberated by the direct cytocidal effect on myocytes or as aberrantly expressed antigens on the surface of virally infected cells. This study demonstrates the

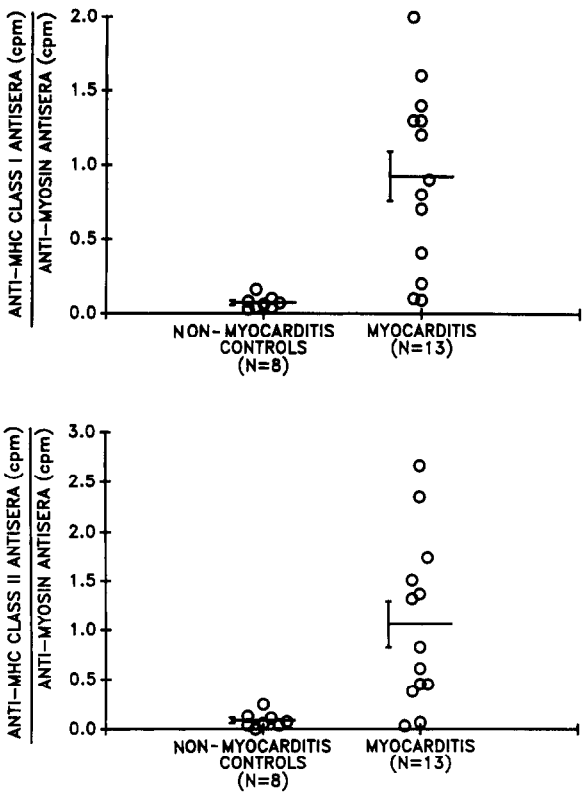

Figure 5. Radioimmunoassay results from individual patients for ratio of MHC class I and class II antigen expression to myosin expression, showing clusters of low ratios for the nonmyocarditis control samples. **Top**, MHC class I ratios; **bottom**, MHC class II ratios. Bars represent mean values ± standard deviations.

induction of MHC class I and class II antigens both within the cardiac interstitium and on myocytes, allowing cardiac antigen or antigens to be recognized by the immune system in the context of self-MHC molecules (17,18). A chronic state of inflammation characteristic of human myocarditis could thus be generated.

Table 3. Specificities, Sensitivities and Predictive Values of Various Combinations of the Significant Immunopathologic Variables That Distinguish Myocarditis Cases From Nonmyocarditis Control Cases

	Specificity (%)	Sensitivity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
A. Counts/min class I MHC*	100	86	100	80
B. Ratio class II counts/min to antimyosin counts/min†	88	79	92	70
C. Counts/min class I and ratio class II	100	71	100	67
D. Counts/min class I or ratio class II	88	93	93	88

*raw counts/min. †ratio of class I and class II counts/min to antimyosin counts/min. MHC = major histocompatibility complex.

The well characterized MHC class I (HLA A, B and C) antigens are found on virtually all nucleated cells. Dual recognition of MHC class I and class II antigens is necessary for activation by cytotoxic T cells for lysis of virus-infected cells (28) and cells bearing alloantigens (18). The MHC class II antigens are involved in communication among cells that regulate the immune response. These antigens are found primarily on B lymphocytes and on antigen-presenting cells, for example, Kupffer cells in the liver, Langerhans cells in the skin, interstitial dendritic cells in the heart (33). Low levels of MHC class II antigens may also be found on capillary endothelium. Normal fetal and adult myocytes express very low levels of MHC class I antigens and do not have detectable levels of MHC class II antigens (30). Endothelial cells lining the microvasculature may express both MHC class I and class II antigens, whereas interstitial dendritic cells (myocardial antigen presenting cells) express only MHC class II antigens (31).

Induction of MHC antigen expression within the myocardium in patients with active myocarditis. This study supports previously described patterns of MHC antigen distribution in normal myocardium and demonstrates two abnormal patterns of induction of MHC antigens in myocardial tissue of patients with active myocarditis: 1) intense induction of MHC class I and class II antigens within the cardiac interstitium (primarily the microvasculature), and 2) abnormal expression of MHC class I and class II antigens on myocyte cell surfaces. Our preliminary findings also suggest that ischemic disruption of myocardial cells as occurs in ischemic heart disease (Group 2, Patients 14 to 18, Tables 1 and 2) does not induce abnormal expression of MHC class I and class II antigens.

Two techniques were used to demonstrate myocardial MHC antigen expression. The widely available immunoperoxidase method localized the cellular distribution of MHC expression (Fig. 2 to 4). Because the limits of sensitivity of this semiquantitative technique are not known, a quantitative radioimmunoassay technique was also used to provide an objective measurement of MHC class I and class II expression within the entire endomyocardial specimen. The immunoperoxidase and radioimmunoassay techniques are significantly correlated with one another for MHC class I and class II expression ($R = 0.911$, $R^2 = 0.830$, $p < 0.001$ and $R = 0.825$, $R^2 = 0.681$, $p < 0.001$, respectively). Therefore, the radioimmunoassay confirmed the subjective impressions of the immunoperoxidase method. When both techniques are compared for their ability to predict an active myocarditis biopsy, logistic regression analysis reveals that the most significant predictors are both derived from the radioimmunoassay; the radioimmunoassay MHC class I counts/min ($p < 0.0005$) and the ratio of MHC class II to myosin content ($p = 0.008$). The immunoperoxidase tech-

nique is ideally suited for localization of MHC staining, whereas the radioimmunoassay is a more sensitive tool to quantitate these immune markers within the myocardium.

Pattern of myocardial MHC expression by immunoperoxidase. Endomyocardial biopsy specimens from active myocarditis patients with high radioimmunoassay counts invariably revealed a strong, diffuse interstitial staining pattern (Fig. 3 and 4) by immunoperoxidase staining. This diffuse increase in MHC induction, particularly within the cardiac microcirculation, may serve as a diffuse tissue marker of immune activation. Myocyte surface staining was focal or multifocal but frequently had equally intense staining characteristics as compared with that of endothelium (Fig. 4c). Of the 11 group I patients with myocarditis and abnormal expression of MHC class I and class II antigens as measured by radioimmunoassay, 8 had foci where definite myocyte surface staining was seen. The possibility that the sarcolemmal staining resulted from peroxidase product spillover from the intensely positive interstitium was considered unlikely, because the staining pattern was always focal (within clusters of myocytes) and not adjacent to strongly stained larger venous and arterial vessels. Two samples from patients with active myocarditis did not stain for abnormal levels of either MHC class I or MHC class II antigens. These patients could not be clinically or histologically distinguished from the other patients with active myocarditis.

Interstitial inflammatory cells were more frequently MHC class I positive than MHC class II positive. MHC class II positive plasma cells were rarely identified. In addition, there appeared to be no consistent change in the intensity of myocardial or microvascular MHC expression adjacent to areas of interstitial inflammation or foci of myocyte necrosis. Although this finding may imply that there is no necessary role for these antigens in the pathogenesis of necrosis, it must be understood that 1) foci of myocyte necrosis frequently involve no more than a few individual myocytes (such focal increased MHC expression could easily be missed unless multiple step sections are examined), and 2) whereas focal and multifocal collections of immune cells are easily identified in human myocarditis, a more subtle generalized increase in interstitial mononuclear cells may also play a role in producing the global increase in MHC antigen induction throughout the myocardium.

Relation of myocardial MHC antigen expression and circulating heart autoantibodies. Another marker of immune activation by cardiac antigens is serologic evidence of circulating heart autoantibodies, which have been previously described in patients with cardiomyopathy and myocarditis (34–36), postpericardiotomy syndrome (37), rheumatic carditis and Chagas' disease (2,3,38,39). Normal volunteers have a 4% prevalence of circulating heart autoantibodies (unpublished data). Group I patients with myocarditis had a 62% prevalence of circulating heart autoantibodies, and none of the eight group II control patients had significant titers of antibodies. Linear regression analysis indicated that tissue-bound MHC antigen expression and serum circulating heart autoantibodies are related. The quantity of MHC class I and class II antigen expression within an endomyocardial biopsy as measured by the radioimmunoassay method correlates well with the titer of circulating immunoglobulin G heart autoantibodies ($r = 0.593$, $p = 0.006$ and $r = 0.708$, $p < 0.001$, respectively). Seven of the eight patients with myocarditis and significant titers of circulating heart autoantibodies had abnormal myocardial MHC class II antigen expression both by immunoperoxidase and by radioimmunoassay.

Clinical implications. We are aware of the limitations in the present study. More cases of active myocarditis need to be studied to determine the true prevalence of increased myocardial and interstitial MHC antigen expression. The precise cellular components expressing abnormal levels of MHC antigens need to be more fully characterized by immunoelectron micrographic techniques. There is also currently insufficient data to comment on the relation between expression of MHC class I and class II antigens and the presence of viral genome within myocardial cells and the relation between a clinical response to immunosuppressive therapy and expression of MHC antigens. In addition, this study only attempted to analyze histologically or clinically distinct categories of disease before attempting to characterize the large, yet undefined group of patients with suspected myocarditis with nondiagnostic endomyocardial biopsies. Nevertheless, there is growing controversy surrounding the sensitivity of the endomyocardial biopsy in identifying patients with active myocarditis on histologic grounds alone. Histologic lesions that characterize most cases of active myocarditis are focal, whereas immune markers such as the MHC antigen expression demonstrated in this study appear to be diffusely expressed throughout the biopsy sample.

Immune markers such as MHC antigen expression on myocytes and microvascular endothelium could be used as a more sensitive tool in identifying patients who have developed an immune-mediated cardiomyopathy. In addition to issues of diagnostic sensitivity, increased MHC expression may be a more specific marker for immune-mediated heart disease than are histologic findings alone. Despite having inflammation and myocyte damage secondary to ischemic heart injury, all five of our ischemic heart disease control samples failed to express increased MHC antigens. Immunoglobulin G deposition can also be easily demonstrated throughout biopsy samples in cases of active myocarditis, but immunoglobulin G deposition appears to be a nonspecific marker of cardiac injury and is frequently very intensely positive in ischemic heart disease control samples (40).

Conclusions. This study demonstrates the increased expression of both major histocompatibility complex (MHC) class I and class II antigens on myocytes and microvascular endothelium in endomyocardial biopsy specimens from patients with active myocarditis. The finding of increased MHC

antigen expression within the myocardium may become a useful adjunct to the histologic diagnosis of myocarditis.

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