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Left Ventricular Mass Increases During Cardiac Allograft Vascular Rejection

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Objectives. **This study evaluated whether left ventricular mass increases during cellular or vascular (humoral) cardiac allograft rejection.**

Background. **An increase in left ventricular mass during cellular cardiac allograft rejection has been described by other investigators, although controversy has existed over the validity of these findings. Left ventricular mass changes have not been evaluated in the setting of vascular (humoral) cardiac allograft rejection.**

Methods. **To determine the effect of allograft rejection on left ventricular mass, we retrospectively reviewed endomyocardial biopsy results and corresponding echocardiograms in 41 cardiac transplant recipients undergoing treatment for allograft rejection. Left ventricular mass was assessed by two-dimensional echocardiography using the method of Schiller. Maintenance immunosuppression included cyclosporine in all patients.**

Results. **Although significant changes in left ventricular wall thickness, mass and dimensions were not observed in patients experiencing moderate or severe cellular allograft rejection (International Society for Heart and Lung Transplantation grades III and IV, n = 27), marked changes were noted in patients with vascular (humoral) rejection (n = 14). Patients with vascular rejection demonstrated an echocardiographic mean (±SEM) increase in left ventricular wall mass (from 109 ± 17 to 151 ± 17 g), and left ventricular wall thickness (from 1.3 ± 0.1 to 1.6 ± 0.1 cm) during the rejection episode. Additionally, vascular rejection was associated with a trend toward an increase in left ventricular systolic dimension (from 2.6 ± 0.1 to 3.0 ± 0.2 cm) and a decrease in left ventricular fractional shortening and increased incidence of hemodynamic compromise with rejection (50% for vascular vs. 11% for cellular rejection).**

Conclusions. **Left ventricular mass increases during episodes of vascular (humoral) rejection, but there is no significant change in left ventricular mass during cellular cardiac allograft rejection.**

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Cardiac allograft rejection is defined by histologic features that include the presence of lymphocytic infiltration of the myocardium with or without myocyte necrosis (1). This histologic pattern is termed cellular rejection. A second form of acute allograft rejection, usually mediated by humoral immune mechanisms, was first recognized in renal transplantation. In cardiac transplantation it is associated with capillary injury without interstitial infiltrates. This "vascular" form of rejection is characterized by evidence of endothelial cell activation and can affect the coronary vessels in cardiac allografts (2). Compared with cellular rejection, vascular rejection is less common and occurs earlier after transplantation (3). Vascular allograft rejection is commonly resistant to standard forms of immunosuppressive therapy, which may result in irreversible allograft dysfunction, markedly reduced allograft survival and reduced patient survival (3–5). Several transplant centers have reported that vascular rejection is associated with mortality rates of 44% to 80% (3,5). In addition, vascular rejection may be associated with a higher incidence of allograft coronary artery disease (6).

There are limited light microscopic findings in vascular rejection that include endothelial cell activation or injury and, rarely, perivascular deposition of leukocytes. Immunofluorescent findings are the mainstay in the diagnosis of vascular rejection and include deposition of complement and immunoglobulin in a vascular distribution as well as evidence of capillary leakage of fibrin (7). Unfortunately, this diagnosis may go undetected because immunofluorescent staining is not routinely performed in most centers. The reason for this is multifactorial: More tissue must be extracted from the endomyocardial biopsy sample, processing of the biopsy sample requires expertise in immunofluorescence, and treatment of vascular rejection is at best imperfect at this time.

Since the early 1980s, its has been reported that left ventricular mass as measured by echocardiography increases in the setting of cardiac allograft rejection (8–11). Unfortunately, this finding is not specific for rejection, and its sensitivity is unknown. The magnitude of change in left ventricular mass may be small because other investigators have not found a consistent increase (10). To date, echocardiographic studies of

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cardiac allograft rejection have focused on cellular rejection defined by the presence of lymphocytic infiltrates and myocyte necrosis. The more recently described vascular rejection has not been evaluated by echocardiography. Our empiric observations suggested that left ventricular wall thickness may increase markedly during a vascular rejection episode. Therefore, we retrospectively analyzed echocardiograms of patients with either vascular or cellular rejection for changes in left ventricular mass.

Methods

The purpose of this study was to measure the changes in left ventricular mass during cardiac allograft rejection and compare the changes observed in cellular versus vascular rejection.

Patients. This was a retrospective study of patients who underwent orthotopic cardiac transplantation between March 1985 and November 1991. To be eligible for study, subjects were required to have had at least one episode of either vascular rejection that required enhancement of their immunosuppressive regimen or moderate to severe cellular rejection (International Society of Heart and Lung Transplantation grades III and IV). In addition, subjects were required to have had echocardiography performed before and during the rejection episodes. The biopsy sample and echocardiogram obtained during the rejection episodes were acquired before treatment for rejection was initiated. All patients had maintenance immunosuppressive therapy that included cyclosporine. Patients received antihypertensive medications as necessary to control hypertension. Of 44 eligible subjects, 3 were excluded because of technically poor echocardiograms, leaving 41 subjects in the study, 27 with cellular rejection and 14 with treated vascular rejection. The mean time between the baseline and rejection studies was 46 days.

Echocardiographic measurements. M-mode, two-dimensional, color and pulsed and continuous wave Doppler examinations were performed using a Hewlett-Packard 1000 ultrasound imaging system with a 2.5- or 5.0-MHz transducer, or both, from standard windows (12). Echocardiograms obtained on the date of endomyocardial biopsy-documented rejection (cellular or vascular) were compared with echocardiograms obtained on a separate control date when no rejection was present so that each patient served as his or her own control. Left ventricular diastolic and systolic dimensions and fractional shortening were measured using standard M-mode techniques (13). Left ventricular wall thickness and mass were measured using an off-line computerized analysis (Hewlett-Packard 1000 ultrasound imaging system) by one observer (E.A.G.) in blinded manner using the technique of Schiller et al. (14). Briefly, left ventricular volume was measured using a truncated ellipsoid model. Left ventricular endocardial volume was subtracted from left ventricular epicardial volume, which yielded the actual volume of the left ventricular muscle. This volume was multiplied by the constant 1.06 to calculate left ventricular muscle mass. At least six cardiac cycles were analyzed for each left ventricular mass measurement (three parasternal short-axis and three apical long-axis views). Ten tapes were resubmitted to the observer in blinded manner to assess reproducibility of the echocardiographic measurements. Variability between individual measurements was 3% for left ventricular thickness and 4% for left ventricular mass. To assess the reproducibility of these measurements over time, we analyzed a control group of 10 subjects for whom echocardiograms were obtained concurrently with two successive biopsy samples that were without cellular or vascular rejection. The average difference between successive measurements of left ventricular mass was 4% (first study 104 ± 4 g, second study 100 ± 4 g).

Diagnosis of rejection. Endomyocardial biopsy was performed by the internal jugular approach with a Stanford-modified Caves bioptome. Three to six endomyocardial biopsy fragments per biopsy procedure were obtained and placed on saline-soaked filter paper and taken immediately to the laboratory, where a small representative portion of one biopsy fragment was frozen in OTC freezing compound for immunofluorescence studies. The remaining pieces were immediately immersed in phosphate-buffered 10% formalin and rapidly processed for histologic evaluation by routine methods. Hematoxylin- and eosin-stained sections and Masson trichrome-stained sections were evaluated for each patient (7).

Cellular rejection was defined by modified Billingham criteria (1). Vascular rejection was defined by finding the combination of prominent endothelial cell swelling or vasculitis, or both, on light microscopy, accompanied by prominent interstitial edema and the vascular deposition of immunoglobulin, complement and interstitial fibrin by immunofluorescence (7). An example of vascular rejection is shown in Figure 1.

Statistical analysis. All data are presented as mean value ± SE. For continuous variables, differences between groups were evaluated by repeated-measures analysis of variance. For noncontinuous variables, differences between groups were compared with the Fisher exact test. Differences were considered significant at $p < 0.05$.

Results

Patient characteristics are presented in Table 1. Patients were similar with regard to age, gender, etiology of heart failure, time after transplantation to occurrence of the rejection episode and presence of a positive donor-specific lymphocyte cross-match. OKT3 sensitization and hemodynamic compromise (defined by the need for intravenous inotropic agents for allograft dysfunction during the rejection episode) were significantly more common in patients with vascular rejection.

M-mode findings are shown in Table 2. There were trends toward an increase in left ventricular systolic dimension and a decrease in fractional shortening in the vascular rejection group. However, when analyzed by repeated-measures analysis of variance, these changes were not significantly different between cellular and vascular rejection groups.

The most notable findings were in the variables measured with two-dimensional echocardiography. Left ventricular wall

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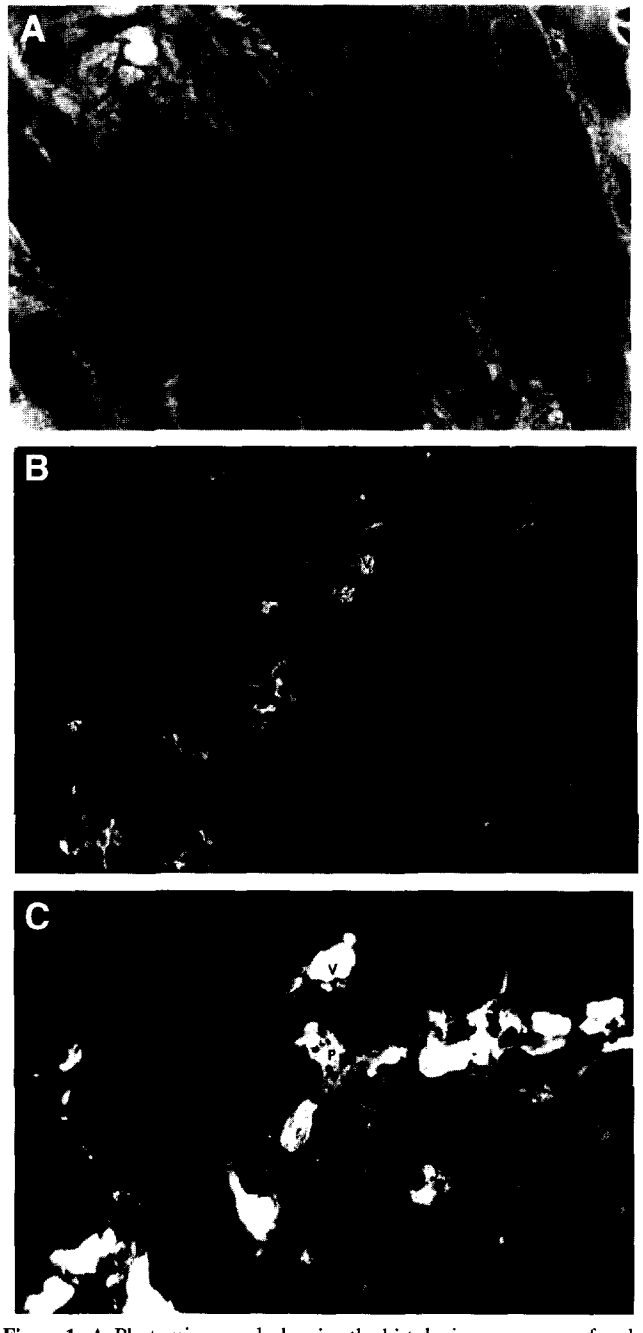

Figure 1. A, Photomicrograph showing the histologic appearance of early vascular rejection. Microvessels have swollen endothelial cells (**arrowheads**), and there is prominent interstitial edema. **B,** Photomicrograph showing immunofluorescent findings in a case of moderate vascular rejection. The tissue was incubated with antibody directed against complement components (C3c). Immunoglobulin G showed identical localization (not shown). Complement deposits (V) are seen as **white staining** and are present only within vessel walls. **C,** Photomicrograph showing immunofluorescent findings in a case of moderate vascular rejection incubated with antibody directed against fibrin, which stains **white**. There is obvious perivascular fibrin staining (P) surrounding fibrin staining of vessels (V). Original magnification ×250, reduced by 51%.

thickness tended to increase, and left ventricular mass significantly increased, in patients treated for vascular rejection (Table 2, Fig. 2); left ventricular wall thickness increased by nearly 20%, and wall mass by nearly 40%, during vascular rejection.

Table 1. Clinical Characteristics of 41 Study Patients

	Cellular Rejection (n = 27)	Vascular Rejection (n = 14)
Age (yr)	39 ± 3	46 ± 4
Gender (M/F)	22/5	10/4
Etiology of heart failure		
CAD	9	7
IDC	16	7
Other	2	
Time after transplantation (mo)	4 ± 1	2 ± 1
Baseline blood pressure (mm Hg)		
Systolic	124 ± 4	119 ± 5
Diastolic	82 ± 3	78 ± 3
Rejection blood pressure (mm Hg)		
Systolic	126 ± 4	120 ± 6
Diastolic	80 ± 2	77 ± 4
Positive cross-match	1	1
OKT3 sensitization	0	3*
Hemodynamic compromise	3 (11%)	7 (50%)*

*$p < 0.05$ versus cellular rejection. Data presented are mean value ± SEM or number (%) of patients. CAD = postinfarction cardiomyopathy; F = female; Hemodynamic compromise = allograft dysfunction requiring intravenous inotropic therapy; IDC = idiopathic dilated cardiomyopathy; M = male.

Discussion

Previous studies. An increase in left ventricular mass in the setting of cardiac allograft rejection is thought to be due to an increase in myocardial interstitial edema or vascular leakage of fibrin, or both (7). However, there may be other reasons for increased left ventricular mass in cardiac transplant recipients, including fluid retention from heart failure or prednisone, left ventricular hypertrophy from hypertension and increased fibrous tissue replacing myocardium following chronic rejection (11). Previous studies of left ventricular mass changes during allograft rejection are quite heterogeneous, with some using M-mode criteria for measurement of left ventricular mass and others using two-dimensional methods. In addition, many of the studies were done before the routine use of cyclosporine (9–11). Some investigators (10,11) have suggested that the

Table 2. Echocardiographic Features of Cardiac Allograft Rejection

	Vascular (n = 14)		Cellular (n = 27)	
	Baseline	Rejection	Baseline	Rejection
LVIDD (cm)	4.4 ± 0.1	4.5 ± 0.1	4.2 ± 0.1	4.3 ± 0.1
LVIDS (cm)	2.6 ± 0.1	3.0 ± 0.2	2.5 ± 0.1	2.6 ± 0.1
LVFS (%)	40 ± 2	34 ± 3	41 ± 2	39 ± 2
LV wall thickness (cm)	1.3 ± 0.1	1.6 ± 0.1	1.4 ± 0.1	1.5 ± 0.1
LV wall mass (g)	109 ± 17	151 ± 17*	95 ± 7	104 ± 7

*$p < 0.05$ vascular versus cellular rejection. Data presented are mean value ± SEM. FS = fractional shortening; IDD = diastolic diameter; IDS = systolic diameter; LV = left ventricular.

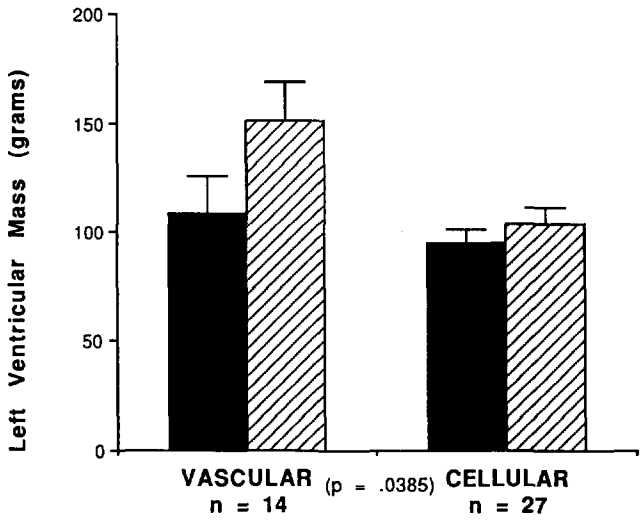

Figure 2. Left ventricular mass at baseline (**solid bars**) and during either vascular or cellular rejection (**crosshatched bars**). Left ventricular mass increased significantly in subjects with vascular rejection compared with those with cellular rejection. The p value for repeated-measures analysis of variance is shown in parentheses along the **abscissa.**

magnitude of change in left ventricular mass during rejection episodes is less with the use of cyclosporine immunosuppression. Dawkins et al. (10), for example, found no change in left ventricular mass using M-mode techniques in one study after the advent of cyclosporine. However, Mastropolo et al. (9) did find a statistically significant increase in left ventricular mass during cellular rejection in 17 patients by means of two-dimensional echocardiographic methods in patients with immunosuppression cyclosporine therapy.

To our knowledge, the effects of vascular rejection on left ventricular mass have not been previously studied. In our subjects left ventricular mass increased during vascular rejection but not during cellular rejection. The typical findings on biopsy of vascular rejection include endothelial cell edema or vasculitis, or both, on light microscopy and the vascular deposition of immunoglobulin and complement by immunofluorescence techniques. These abnormalities may be responsible for the observed increase in wall thickness either directly or indirectly by altering capillary permeability and thus increasing interstitial fluid, leading to deposition of interstitial fibrin. Several investigators (15,16) noted an increase in interstitial and vascular fibrin on immunocytochemical testing. Labarrere et al. (15) showed that these findings are always associated with a loss of endothelial antithrombin III in arterioles and venules and a loss of arteriolar smooth muscle tissue plasminogen activator. These findings can be detected early in the posttransplant period and are associated with clinically significant cardiac compromise and were confirmed by Hammond et al. (16).

Clinical implications. The echocardiographic manifestations of vascular rejection may be of considerable clinical importance. An increase in left ventricular mass detected by echocardiography may suggest the presence of vascular rejection. However, our data require further verification in a larger population and a prospective evaluation.

Paulsen et al. (17) first demonstrated that echocardiographically derived indexes of diastolic function were abnormal in patients with acute allograft rejection. Since that time, Valantine et al. (18,19), Haverish et al. (20) and Desruennes et al. (21) have demonstrated that Doppler indexes of diastolic function were abnormal in patients with rejection. In all these studies, a 5% to 20% incidence of false positive results has been noted. Although these "false positive" results are normal by cellular criteria on the endomyocardial biopsy, such patients may have undiagnosed vascular rejection because most centers do not routinely perform surveillance for vascular rejection. We advocate evaluation for vascular rejection in these patients by endomyocardial biopsy and measurement of left ventricular mass.

Study limitations. The present study has the usual limitations of retrospective evaluations. The possible effects of sampling errors from endomyocardial biopsy on our findings are unknown. The number of cardiac allograft recipients with vascular rejection that required treatment was limited. However, we did evaluate all patients treated for vascular rejection at our institution who had adequate data for review. The vascular rejection group had a significantly higher incidence of allograft dysfunction, resulting in hemodynamic compromise. It is possible that our results reflect a decrease in allograft function, not differences between the types of rejection. However, the changes in left ventricular mass of patients with vascular rejection without hemodynamic compromise also were greater than changes observed with cellular rejection with hemodynamic compromise (data not presented). In addition, more patients with vascular rejection than those with cellular rejection became sensitized to OKT3. This finding is expected because OKT3 sensitization is strongly associated with vascular rejection (4).

Conclusions. Vascular rejection is associated with a significant increase in left ventricular mass that is not observed with cellular rejection. The significance of these changes is uncertain, but they suggest a role for alterations in vascular permeability in this disorder.

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