Functional Significance of Intimal Thickening as Detected by Intravascular Ultrasound Early and Late After Cardiac Transplantation

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**Background.** Detection of transplant coronary disease remains difficult. Both intravascular ultrasound (IVUS) imaging and functional vascular function studies have been used to evaluate this process. However, the time course of intimal thickening as assessed by IVUS and the relation between structure and function have not been explored.

**Methods and Results.** In 40 patients 1 to 8 years after transplantation, 108 coronary artery segments were analyzed by IVUS. Intimal index [% intimal area (lumen-intimal area)] and maximal thickness were used to quantify intimal thickening. Abnormal IVUS was present in 53 of 108 segments (49%) (mean intimal index of diseased segments, 23±2%; maximal thickness, 530±47 µm). For those patients with intimal thickening in all segments of the analyzed artery, more time had elapsed since transplantation (4.3±0.6 years) than for those whose arteries contained some normal (2.6±0.3 years) or all normal segments (2.2±0.6 years, P<0.05). Both the proportion of segments with intimal thickening and the degree of thickening increased as a function of time after transplantation (P<0.5). By multivariate analysis, the independent predictors of intimal thickening were increasing time after transplantation and pretransplantation hypercholesterolemia (P=0.02). Within the cohort of 40 patients, endothelium-dependent vasomotor function was evaluated in 26 matched segments from 11 patients studied 1 year after transplantation and in 15 matched segments from 8 patients studied ≥5 years after transplantation by serial infusions of acetylcholine (10–8 to 10–6 mol/L). Of the 26 segments assessed for structure/function correlation at 1 year after transplantation, 22 had no intimal thickening by IVUS. However, endothelial dysfunction was present in 13 of these normal segments (mean diameter constriction, 18.8±2.3%). Of the 15 segments studied ≥5 years after transplantation, 11 had intimal thickening. None of these 11 segments had preserved endothelial function (mean diameter dilation, 8.6±2.9%). There was no relation between the degree of intimal thickening and the magnitude of the endothelium-dependent response to acetylcholine.

**Conclusions.** This study has shown that intimal thickening after transplantation begins as a heterogeneous process and increases in extent and magnitude over time. Also, endothelial dysfunction occurs early before the intimal thickening; yet in those patients surviving ≥5 years, endothelial function may recover even in the presence of moderate intimal pathology. The variable relation between intimal pathology and endothelial function is probably a result of the episodic nature of immune injury. (Circulation. 1993;88:1093-1100.)

**Key Words** • endothelium • acetylcholine • transplantation • coronary artery disease

The development of coronary artery disease remains the leading cause of morbidity and mortality in the long-term survival of patients after cardiac transplantation.1–4 Despite intense basic and clinical investigations, the pathogenesis of this accelerated atherosclerotic process remains unclear, but a number of clinical and immunological factors have been implicated.5–10 These factors include cytomegalovirus seroconversion, diabetes, hypercholesterolemia, and histocompatibility mismatch.6 The most plausible pathophysiological mechanism is the exposure of donor antigens to the recipient's immune system, leading to activation of adhesion molecules and various cytokines in a milieu of hypercholesterolemia and other traditional risk factors, thus progressively leading to smooth muscle cell proliferation and collagen deposition.11

The clinical detection of transplant coronary disease relies principally on coronary angiography, which demonstrates that up to 50% of the patients will develop significant angiographic disease by 5 years after transplantation.2,12 However, given the diffuse nature of the disease, angiography is usually unable to detect early changes until focal lesions have developed or severe distal pruning of the vessels has occurred. Recently, a new imaging modality, intravascular ultrasound (IVUS), has been used to better define the early intimal changes before angiographic changes have occurred. Pinto et al13 and St. Goar et al14 have shown that intimal
thickening that is angiographically silent can be detected by IVUS and that this occurs in a majority of patients after transplantation. However, the functional significance of this intimal thickening is not known.

Accumulating evidence suggests that the vascular endothelium becomes dysfunctional in the early stages of native coronary atherosclerosis. Normal coronary arteries with intact endothelium vasodilate in response to the endothelium-dependent agonist acetylcholine, whereas atherosclerotic coronary arteries invariably vasoconstrict, demonstrating the presence of endothelial dysfunction. Similar observations have been made in cardiac transplant patients, with a large proportion of patients showing endothelial dysfunction by the first year after transplantation even though angiographically the arteries appear smooth. However, it is not clear whether this dysfunction is caused by the presence of angiographically silent intimal thickening or whether the dysfunction can occur in morphologically normal arteries. In this study, we investigated the relation between intimal thickening as evaluated by IVUS and endothelial function as tested by acetylcholine both early and late after transplantation. Our hypotheses are that (1) endothelial dysfunction occurs in the early stages of transplant coronary disease preceding structural changes as seen by IVUS and (2) the prevalence and severity of the intimal pathology increases with time and is associated with worsening endothelial function in those studied late after transplantation.

Methods

Patient Population

This is a cross-sectional study consisting of 44 cardiac transplant recipients who were studied once at routine annual evaluation (years 1 to 8) from April 1991 through February 1992. During this period, a total of 85 patients presented for annual evaluation. Forty-one patients were not studied because of the presence of significant peripheral vascular disease, failure to give informed consent, and logistic reasons in the catheterization laboratory. The clinical and angiographic characteristics of these 41 patients excluded were not different from those of the study group. Within the total cohort of study patients, 11 patients were studied at 1 year and 8 patients were studied at ≥5 years after transplantation. The data from these 19 patients were used for comparison between endothelial functional testing and structural assessment. All patients were treated with triple immunosuppression (cyclosporin, prednisone, and azathioprine), and no patients had ongoing rejection requiring treatment at the time of the study.

The clinical characteristics for each patient were obtained, including the ages of the recipient and donor, the diagnosis before transplantation, cardiac risk factors before transplantation, years after transplantation, ischemic time of the heart, cholesterol values at each annual evaluation and a mean cholesterol over that time period, posttransplantation hypertension or diabetes, medical treatment of hypercholesterolemia after transplantation, and the number of treated rejection episodes.

Study Protocol

Written informed consent was obtained from patients before the catheterization procedure in accordance with the guidelines established by the Committee for the Protection of Human Subjects. Long-acting vasoactive medications, including calcium channel blockers, \( \beta \)-blockers, nitrates, and converting enzyme inhibitors, were discontinued for at least 18 hours before the catheterization. After diagnostic right and left heart catheterization and right ventricular biopsy, patients were eligible for the study if there were no stenoses >50% in the left anterior descending artery. The study protocol was performed in 44 patients, but in 2 patients the left anterior descending artery was felt to be too small for passage of the IVUS catheter, and in 2 patients no ultrasound images were obtained because of technical failure. The study population thus consisted of 40 patients (32 men and 8 women).

Intracoronary infusions. After an 8F guiding catheter was positioned in the left main artery and 10,000 units of heparin was infused, a 2.5F infusion catheter was advanced through the guiding catheter over a 0.014-in guide wire into the proximal portion of the left anterior descending artery. Serial intracoronary infusions were made according to an established protocol: (1) control infusion (dextrose 5% in water [D,W]); (2) serial 2.5-minute infusions of the endothelium-dependent vasodilator acetylcholine (Miochol, Iolab Pharmaceuticals, Claremont, Calif) with final estimated intracoronary concentrations of 10<sup>-4</sup>, 10<sup>-5</sup>, and 10<sup>-6</sup> mol/L; (3) 5-minute recontrol infusion of D,W; and (4) 3-minute infusion of the endothelium-independent vasodilator nitroglycerin at 16 mg/min. During each infusion, the blood pressure, heart rate, and ECG were continuously monitored.

Quantitative coronary angiographic images were taken after each intervention by a previously validated method. A nonionic contrast medium (Omnipaque, Winthrop Laboratories, New York, NY) was injected into the left coronary artery at 7 mL/s for a total of 9 mL with a power injector (Mmedrad, Pittsburgh, Pa) to opacify the coronary artery. The study vessel was positioned in the center of the screen such that overlap of adjacent arteries was minimized.

IVUS. After the above infusion system was removed, the intracoronary imaging system was passed into the left anterior descending artery over a 0.014-in guide wire. Sublingual nitroglycerin (0.4 mg) was given before insertion of the catheter. The imaging system consisted of a 30-MHz transducer and rotating mirror enclosed within an acoustic housing at the tip of a 5F or 4.3F, 135-cm-long catheter (CVIS Inc, Sunnyvale, Calif). At the focal depth, the axial resolution of this system is estimated to be 150 μm, and the lateral resolution is 200 μm. The radius of penetration is 4 to 5 mm.

The imaging catheter was advanced as distally as possible into the left anterior descending artery. Images were obtained at regular intervals with adjustment of the time-gain-control and reject level to obtain the optimum images. The image positions were at least 1 cm apart, and three positions were obtained for each patient. The position of the ultrasound catheter in the artery was recorded by cineangiography after the injection of contrast, and the position was measured from the nearest landmark (ie, side branches). After image acquisition, the catheter was readvanced to the most distal position, and then the catheter was pulled back, examining the whole length of the artery.
Analysis

**IVUS measurements.** IVUS images were recorded on S-VHS tape and digitized into a Macintosh computer for quantitative analysis (University of California, San Francisco; Intravascular Ultrasound Research Group Software). Twelve of the total of 120 image positions (three images per patient \( \times 40 \) patients) were of suboptimal quality and were not analyzable. The ultrasound images at each position were interpreted by two observers blinded to the results of the quantitative angiography. The observers had access to the S-VHS recording as well as the digitized image. The lumen/intima interface was traced by planimetry, and if measurable intimal thickening was present, the outer border of the thickening was also planimetered (intima/media border, Fig 1). The intimal thickening was measured at eight radial chords, and the maximal thickness was recorded. The angle subtended by the thickening (angle of atherosclerosis) and the major and minor diameters of the lumen were also measured. An index of intimal thickness was calculated as \( \text{intimal area}/(\text{lumen}+\text{intima area}) \). The images were also analyzed qualitatively for the presence of calcium. The results of the three digitized frames at each position were averaged to obtain the data for that position.

Segments were classified as abnormal if measurable intimal thickening was present (>150 \( \mu m \)) or normal if no thickening was present. For the purpose of comparison, patients were also categorized into three groups on the basis of the IVUS images: group 1, all segments normal; group 2, heterogeneous, if the patient had normal and abnormal segments; group 3, all segments abnormal. The above classification is based on all the analyzable ultrasound segments in each patient as well as the images recorded during pullback of the ultrasound catheter.

**Quantitative coronary angiography.** Technically suitable single-plane angiograms were selected for computer analysis on the basis of a previously described method.\(^{15,17,19,22}\) Two to three segments 5 mm in length were selected for analysis prospectively on the basis of optimal regions for quantitative angiographic analysis. Segments had to be in nonoverlapping or nonbifurcating regions and at least two vessel diameters distal to the infusion catheter to ensure adequate mixing of the infusates. Segments were not selected on the basis of vasomotor responses to acetylcholine. The diameters after the control and recontrol infusions were used as references to calculate the percent diameter change to the serial doses of acetylcholine and nitroglycerin, respectively.

For the comparison of endothelial function and structure by IVUS, 26 segments from 11 patients 1 year after transplantation and 15 segments from 8 patients \( \geq 5 \) years after transplantation were evaluated. These segments had quantitative angiographic assessment of coronary vasomotion and contained analyzable ultrasound positions. Only quantitative angiographic measurements from the immediate region (4 to 5 mm) straddling each ultrasound position were included for the assessment of endothelial function. This allowed matching of structure and function and coronary vasomotion as closely as possible. The sites of correlation were at least 1 cm apart when more than one were included from the same artery. If possible, sites from both proximal and distal portions of the artery were included for analysis.

**Statistics**

Relations between clinical characteristics, acetylcholine response, and IVUS parameters and angiographic results were explored by either ANOVA (for categorical variables) or linear regression (for continuous variables). The analyses were performed with the Generalized Estimating Equations program for SAS (SAS Institute Inc, Cary, NC). This allowed the calculation of regression coefficients for multiple arterial segments per patient that may not be independent (see "Appendix"). Determinants of intimal thickening by IVUS and acetylcholine response were calculated by multivariate regression analysis. Statistical significance was defined as a two-sided \( P \) value of \( P < .05 \). All data are expressed as mean±SEM.
TABLE 1. Clinical Characteristics of the Study Patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>9</td>
<td>19</td>
<td>12</td>
</tr>
<tr>
<td>No. of segments</td>
<td>27</td>
<td>52</td>
<td>29</td>
</tr>
<tr>
<td>Donor age (y)</td>
<td>20.2±2.3</td>
<td>27.5±2.3</td>
<td>30.0±2.9</td>
</tr>
<tr>
<td>Recipient age (y)</td>
<td>47.9±4.2</td>
<td>45.7±2.4</td>
<td>46.5±3.7</td>
</tr>
<tr>
<td>CMP/CAD</td>
<td>6/3</td>
<td>15/4</td>
<td>10/2</td>
</tr>
<tr>
<td>Time after Tx (y)</td>
<td>2.2±0.6</td>
<td>2.6±0.3</td>
<td>4.3±0.6*</td>
</tr>
<tr>
<td>Donor ischemic time (min)</td>
<td>166±16</td>
<td>171±15</td>
<td>148±13</td>
</tr>
<tr>
<td>Mean cholesterol (mg/dL)</td>
<td>237±11</td>
<td>229±9</td>
<td>241±17</td>
</tr>
<tr>
<td>No. of treated rejection episodes</td>
<td>1.1±0.5</td>
<td>1.3±0.3</td>
<td>1.3±0.3</td>
</tr>
<tr>
<td>Intimal index (%)</td>
<td>0</td>
<td>7±2</td>
<td>29±5†</td>
</tr>
<tr>
<td>Maximal thickness (μm)</td>
<td>0</td>
<td>190±62</td>
<td>620±87†</td>
</tr>
<tr>
<td>Angle subtended by intimal thickening (degrees)</td>
<td>0</td>
<td>89±27</td>
<td>257±29†</td>
</tr>
</tbody>
</table>

Group 1, patients with all normal intravascular ultrasound (IVUS) segments; group 2, patients with both normal and abnormal IVUS segments; group 3, patients with all abnormal IVUS segments. CMP, cardiomyopathy; CAD, coronary artery disease; Tx, transplantation. Values are mean±SEM. *P<.05; †P<.001.

Results

Clinical Characteristics

The mean age of the 40 study patients was 46 years (range, 16 to 61 years), and the mean age of the donors was 27 years (range, 11 to 52 years). Patients were studied at a mean of 3.0 years (range, 1 to 8 years) after transplantation. The pretransplantation diagnosis was cardiomyopathy in 31 patients (77.5%) and coronary artery disease in 9 (22.5%). The pretransplantation cardiac risk factors included diabetes mellitus in 4 patients (10%), hypertension in 9 (22.5%), cigarette smoking in 18 (45%), and hypercholesterolemia in 9 (22.5%). After transplantation, 27 patients (67.5%) were on lovastatin for hypercholesterolemia, and 22 (55%) had hypertension requiring treatment. The mean cholesterol after transplantation for the group was 235±7 mg/dL. Four patients had noncritical stenoses, and 18 patients had minor irregularities on angiography.

IVUS

The majority of these patients had angiographically smooth coronary arteries, although 4 patients had stenoses of <50%. A total of 108 segments were analyzed by IVUS in the 40 patients. A wide range of pathological lesions was seen among the patients studied, ranging from no intimal thickening to >1 mm of concentric intimal thickening. Calcification was present in only 3 of 108 segments. The clinical characteristics for the three groups, based on the overall ultrasound appearance, are shown in the Table. In patients whose arteries showed all abnormal ultrasound segments (group 3), more time had elapsed after transplant than in those in the other two groups (P<.05). There was also a trend for these patients to have older donors (P=.06). As expected, group 3 patients had a greater burden of intimal thickening as assessed by maximal intimal thickening, angle of atherosclerosis, and intimal index than the heterogeneous group (group 2, P<.0001). There was no difference in the intimal index and maximal thickness between those segments studied in the proximal portion of the vessel and those in the middistal vessel.

Fig 2 shows the intimal index and maximal thickness of the 108 segments plotted as a function of years after transplantation. The burden of disease was seen to increase steadily over time. The proportion of segments that were abnormal was small at year 1 (6 of 30, 20%) and increased over time (15 of 19, 79% at ≥5 years; P<.05).

Univariate regression analysis (corrected for multiple segments per patient) identified time after transplantation, a pretransplant history of hypercholesterolemia, and increased mean cholesterol after transplantation as significant factors associated with intimal thickening. Other factors, including rejection episodes, donor ischemic time, donor age, and diabetes mellitus, were not associated with intimal thickening. Multivariate analysis identified a history of hypercholesterolemia before transplantation and years after transplantation as the independent predictors of increased intimal index.

Structure and Function Analysis, Year 1 Patients

Eleven patients who were 1 year after transplantation had 26 segments matched for structure/function analysis. All had normal angiograms. Intimal thickening was present in at least one segment of the coronary arteries in 3 patients, and intimal thickening was completely absent in 8 patients. Of the 22 segments that had no intimal thickening, 13 demonstrated endothelial dysfunction with vasoconstriction to acetylcholine (mean diameter change, -18.8±2.3%; Fig 3), and 9 had retained endothelial function (mean diameter change, 6.3±1.8%). Four of 26 segments had intimal thickening, and all 4 segments (intimal index, 18±7%; maximal thickness, 0.24±0.1 μm) demonstrated the presence of endothelial dysfunction with a mean diameter change of -21.5±2.0%. The response to the endothelium-independent vasodilator nitroglycerin was retained in all segments (mean diameter change, 25.0±2.3%). There were no clinical or ultrasound characteristics that predicted the acetylcholine response.

Structure and Function Analysis, Year 5 and Beyond

Eight patients who were ≥5 years after transplantation had 15 segments matched for structure/function
analysis. Three of the patients had noncritical stenoses in the circumflex or right coronary arteries and mild tapering in the left anterior descending artery. Intimal thickening was present in at least one portion of the coronary artery in seven patients, and only one patient had no intimal thickening at all. Only 4 of 15 segments were free of intimal thickening. One of these segments demonstrated endothelial dysfunction with a \(-14.8\%\) constriction to acetylcholine, but the other 3 segments had retained endothelial function (mean diameter change, \(8.3\pm0.5\%\)). Eleven of 15 segments had intimal thickening (intimal index, \(34\pm6.6\%\); maximal thickness, \(0.69\pm0.11 \mu m\)), and 2 demonstrated endothelial dysfunction (mean diameter change, \(-30.2\pm9.1\%\)). Surprisingly, the majority (9 segments) with intimal thickening had preserved endothelial function to acetylcholine testing (mean diameter change, \(8.6\pm2.9\%\); Fig 4). There was no difference in the burden of atherosclerosis by IVUS in those segments with constriction vs those with dilation to acetylcholine. No clinical or ultrasound characteristic predicted the acetylcholine response. In particular, there was no difference in the functional response in those segments with eccentric thickening vs those with concentric disease. There was a trend for less dilation to nitroglycerin in those patients studied late after transplantation compared with those studied at 1 year (\(P=0.10\)). However, there was no correlation between the degree of diameter change to acetylcholine and the response to nitroglycerin.

Fig 5 summarizes the structure/function relation at year 1 and at \(\geq 5\) years after transplantation. In patients 1 year after transplant, most (17 of 26, 65\%) of the coronary segments had endothelial dysfunction, espe-

FIG 3. Intravascular ultrasound image in a coronary segment without intimal thickening at year 1 demonstrates vasoconstriction to endothelium-dependent vasodilator, acetylcholine (Ach), and preserved endothelium-independent vasodilator, nitroglycerin (TNG).

FIG 4. Intravascular ultrasound images in coronary segments with (A) eccentric intimal thickening (intimal index, 26\%; maximal thickness, 540 \(\mu m\)) and (B) concentric intimal thickening (intimal index, 46\%; maximal thickness, 740 \(\mu m\)) at \(\geq 5\) years after transplant. These segments demonstrate intact endothelium-dependent vasodilation to acetylcholine (Ach) and endothelium-independent vasodilation to nitroglycerin (TNG).
specialiy when intimal thickening was present (4 of 4, 100%). In patients who survived ≥5 years after transplantation, the large majority of the coronary segments had preserved endothelial vasodilator function (12 of 15, 80%) regardless of whether intimal thickening was present by IVUS.

Discussion

As expected, this study demonstrated that the degree of intimal thickening as detected by IVUS appears to increase steadily with time after transplantation and that endothelial dysfunction may precede intimal thickening early after transplantation.

This study also demonstrated that preserved endothelial vasodilator function may be seen in patients surviving ≥5 years after transplantation who are well clinically, even in the presence of intimal thickening. Thus, our hypotheses that endothelial function would be worse in those coronary segments with increasing intimal thickening and that preservation of endothelial function would not be possible in these coronary segments is refuted in this transplant population.

IVUS

Both in vitro and in vivo studies have confirmed the utility of IVUS in the assessment of coronary pathological lesions. In native-vessel atherosclerosis, intimal pathological lesions as detected by IVUS were found to precede changes seen by coronary angiography.23 Similarly, several groups have shown that IVUS was more sensitive than angiography in the detection of intimal thickening in cardiac transplant patients.13,14,24,25

In contrast to a previous report showing that all patients at 1 year had at least minimal thickening,14 this study shows that only a minority of patients (3 of 11; 27%) or coronary segments (6 of 30; 20%) studied at 1 year demonstrated intimal pathological lesions. This difference is probably a result of the method of classification. In the previous study, patients were classified according to the most severe of four segments, which may overestimate the involvement on a per-patient basis. Thus, intimal disease may be present at year 1 after transplantation, but the degree of involvement is probably low. Not unexpectedly, the prevalence and severity of intimal thickening are found to increase over time after transplantation, as suggested by recent preliminary reports.25,26

The present data also suggest that transplant coronary disease is a heterogeneous process. Early after transplantation, there is little if any thickening in the coronary vessels. With time, patchy thickening begins to develop throughout the vessel, with no preference for distal or proximal segments. As the disease progresses, all segments of the artery become involved. Routine angiography is unable to detect these changes (90% of the study population has smooth coronary arteries), since the thickening can be as little as 200 μm and concentric in involvement.

Previous studies based on angiography have failed to consistently identify factors associated with transplant atherosclerosis. Since IVUS is more sensitive, it is likely that correlates of intimal thickening would be more accurately identified and more consistent. The only previous correlate reported has been the total amount of steroid used in a group of 21 patients studied with IVUS.27 In the present study, we found by multivariate analysis that the time after transplantation and a history of pretransplantation hypercholesterolemia were independently correlated with intimal thickening. Patients with a history of hypercholesterolemia before transplantation may be prone to develop posttransplantation hypercholesterolemia (univariate correlate) and may also have unidentified systemic factors that predispose them to atherosclerosis.

Endothelial Function and Its Relation to IVUS Findings

Angiographic studies of patients with native-vessel atherosclerosis have shown that endothelial dysfunction may occur in smooth coronary arteries, and this may be related to risk factors for atherosclerosis.15,16 However, it is not clear whether this occurs because of occult intimal thickening undetected by angiography or whether endothelial function is impaired without structural abnormalities. The present study clearly shows that endothelial function indeed can be impaired without the presence of intimal pathological lesions in transplanted coronary arteries at one year after transplantation. This observation is supported by organ bath experiments showing that the addition of oxidized low-density lipoprotein cholesterol to arterial rings alone can lead to endothelial dysfunction.26 Thus, other factors besides the physical presence of atherosclerosis are important in impairment of the endothelial vasodilator function. At the subcellular level, the mechanisms are not known but may include alterations in the G-protein-dependent pathways of the endothelium.28

Interestingly, many coronary artery segments had preserved endothelial function at 5 years despite moderate intimal thickening in the majority, although it was largely angiographically silent. Several possible explanations exist: (1) A selection bias may have skewed the results toward the more favorable patterns observed in the long-term survivors. The rate of progression in intimal thickening may have been substantially less than
in those patients who succumbed to graft atherosclerosis at an earlier stage, who would not be represented in this cross-sectional study. Despite the presence of cumulative intimal thickening in latter years, preserved endothelial function in these patients may be protective and retard atherosclerosis progression. (2) The coronary artery in later years may be less capable of constriction as a result of fibrosis. This is supported by the finding that arteries that constricted less to acetylcholine also tended to dilate less to nitroglycerin. However, this correlation was weak. Others have shown by IVUS that there is no relation between the nitroglycerin response to time after transplantation and intimal thickness.13

There was no clear relation between the degree and characteristics of the intimal thickening and the acetylcholine response. This seemingly paradoxical behavior might be explained by the episodic nature of immune injury and subsequent recovery, thus raising the possibility of reversing endothelial dysfunction even in the presence of intimal thickening. Confirmation of this hypothesis must await the identification of better markers of immune activity.

**Study Limitations**

Although every effort was made to match the functional testing with the structural analysis by IVUS, we are not certain whether the image from a single position (beam width, 0.25 mm) is an accurate quantitative representation of the whole 5 mm segment. However, given that we carefully interrogate each position back and forth and the ultrasound catheter moves 1 to 2 mm with each cardiac cycle, we are qualitatively certain that the single image is representative of that 5 mm segment. Thus, the comparison of the categorical response of the artery and presence or absence of intimal thickening is not in doubt, but the quantitative burden of disease may not be uniform over the entire 5 mm length used to assess vasomotion. The length of thickening in a particular region may correlate better with function, but because of the intrinsic difficulty of accurately measuring this length by ultrasound, we are unable to provide this correlation.

The findings in this study are based on examination of the proximal one half to two thirds of the left anterior descending artery. Other arteries of the coronary tree were not assessed. Because of the diffuse nature of transplant atherosclerosis, it is likely that the other arteries will exhibit similar behavior.29 Segments that were too distal in the left anterior descending artery were not studied because of safety concerns.

The definition of an abnormal ultrasound appearance was based on the resolution of the imaging system. Previous in vitro studies have demonstrated that the intima in a normal coronary vessel is not seen by IVUS and that a three-layered appearance already represents intimal thickening.21 Studies by our own group in patients studied immediately after transplantation have shown no intimal thickening in the majority of patients.30 However, we acknowledge that the definition of normal is not known with certainty.14

Although this study examined the relation of structure and function in patients at different times after transplantation and was useful in defining the range of responses, it must be remembered that this was a cross-sectional, not a longitudinal, study. It can be assumed that intimal thickening is a patchy and progressive process and that early endothelial dysfunction may be important in initiating the atherosclerotic process, but care must be taken in the interpretation. A longitudinal follow-up study of ultrasound appearance and acetylcholine responses currently under way will be helpful in clarifying these issues.

**Conclusions**

IVUS is a useful method of determining the extent of intimal pathological lesions that are not seen by angiography. Clinical factors, including the length of time after transplantation and pretransplantation hypercholesterolemia, are related to the degree of intimal thickening. Endothelial dysfunction may be seen before structural changes by IVUS, and the vasodilator function may be preserved even in those patients with concentric intimal thickening. This suggests that an episodic endothelial injury, most likely immune in nature, may lead over time to intimal thickening. Patients who are long-term survivors may have a favorable outcome despite intimal thickening because of preserved endothelial function. Future longitudinal studies using IVUS and endothelial function testing may help to further our understanding of the pathophysiology of accelerated atherosclerosis and define better means of earlier detection of this disease.

**Appendix**

The Generalized Estimating Equations program for SAS allows specification of the distribution of the outcome measure as either normal (ie, changes in response to acetylcholine), binary (ie, constriction vs no change/dilation), or gamma (ie, intimal index). Specifically, ANOVA was used to compare clinical characteristics and acetylcholine and IVUS parameters between groups. Differences in the acetylcholine, nitroglycerin, and IVUS characteristics at various times after transplantation were also calculated by ANOVA, using the 1-year data as baseline. The overall trend was determined by regression analysis, treating normal, heterogeneous, and abnormal as ordered classes.

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