Loss of the Coronary Microvascular Response to Acetylcholine in Cardiac Transplant Patients

Charles B. Treasure, MD; Joseph A. Vita, MD; Peter Ganz, MD; Thomas J. Ryan Jr., MD; Frederick J. Schoen, MD, PhD; Vladimir I. Vekstein, MD; Alan C. Yeung, MD; Gilbert H. Mudge, MD; R. Wayne Alexander, MD, PhD; Andrew P. Selwyn, MD; and R. David Fish, MD

Background. The coronary arteries of transplanted hearts frequently develop accelerated diffuse arteriosclerosis. The effects of this disease on resistance vessel function are unknown.

Methods and Results. To investigate the integrity of endothelium-dependent small-vessel vasodilation in transplanted hearts, coronary blood flow (CBF) responses to the endothelium-dependent dilator acetylcholine (10^{-8} to 10^{-6} M) and the essentially endothelium-independent dilator adenosine (10^{-6} to 10^{-4} M) were assessed in 40 studies of 29 transplant patients 1-3 years after transplantation and in seven nontransplanted controls. CBF was measured at constant arterial pressure with a Doppler catheter in the left anterior descending coronary artery. Controls, year 1 transplant patients, and year 2 transplant patients had similar increases in CBF in response to acetylcholine (232±40%, 200±41%, and 201±54%, respectively; p=NS), whereas year 3 transplant patients had increased CBF of only 100±39% (p<0.05 versus controls). An index of the proportion of CBF reserve attributable to endothelium-dependent dilation was obtained by normalizing each patient’s peak acetylcholine flow response by the peak adenosine flow response. In patients receiving both acetylcholine and adenosine, endothelium-dependent flow responses declined over time [57±9% in controls, 56±10% for year 1, 47±12% for year 2, and 29±9% for year 3 (p<0.05 versus controls)]. An increased mean cyclosporine level (range, 99-261 ng/ml) (r=0.67, p=0.004) and increased transplant recipient age (range, 20-63 years) (r=0.51, p=0.004) predicted a preserved endothelium-dependent microvascular response.

Conclusions. Thus, microvascular endothelium-dependent dilation deteriorates over time in the transplanted heart, which may reflect underlying graft arteriosclerosis and contribute to ischemic damage of the myocardium. (Circulation 1992;86:1156-1164)

KEY WORDS • arteriosclerosis • heart transplantation • coronary vessels

A rteriosclerosis in the transplanted heart is an accelerated process that is the most important limiting factor in the long-term survival of cardiac transplant patients. It is a diffuse, concentric process affecting both large and small coronary vessels and is difficult to detect by conventional angiography or noninvasive techniques.

Accelerated transplant arteriosclerosis leads to occlusive vascular changes and subsequent ischemic myocardial damage, as does atherosclerotic coronary artery disease. However, small-vessel disease is more prominent in the transplanted heart. These small intramyocardial vessels have vasomotor regulatory control over coronary blood flow. Dystrophic or dysfunctional changes in these crucial sites of regulation may compromise myocardial perfusion. Thus, these resistance vessels may have a distinctive role in the pathophysiology of graft injury, which may occur in advance of angiographic evidence of disease in the epicardial coronary arteries.

Recently, the endothelium has been recognized as being an important regulator of underlying vasomotor tone in both large and small coronary vessels (via release of endothelium-derived relaxing factor). It has also been shown to be capable of regulating the growth of underlying smooth muscle. The endothelial cell is the first cell type encountered by the bloodborne cells of the host immune system. Thus, the small-vessel endothelium may have a unique role in transplant arteriosclerosis as an early target of immune injury. Potentially, both the proliferative response of vascular smooth muscle and intima (leading to occlusive disease and myocardial ischemia) and vasomotor response (leading to disordered blood flow regulation) may be affected, contributing to ischemic injury.

In humans, both atherosclerotic coronary disease and transplant arteriosclerosis impair large coronary artery vasodilator responses to endothelium-dependent agents. Fish et al used the endothelium-dependent vasodilator acetylcholine to show that epicardial coro-
nary artery endothelium is dysfunctional in most patients 1 year after cardiac transplantation. No studies have evaluated the effect of transplant arteriosclerosis on the microvascular endothelium.

We investigated the hypotheses that 1) the coronary microvascular endothelium is functionally damaged in cardiac transplant patients, and 2) this functional impairment worsens as graft arteriosclerosis progresses.

We tested these hypotheses by evaluating coronary blood flow (CBF) responses to the essentially endothelium-independent dilator adenosine and to the endothelium-dependent vasodilator acetylcholine and correlated this measure of functional endothelial integrity with clinical and laboratory factors.

Methods

Patient Population

Twenty-nine transplant patients underwent 40 studies at 1 (n=17), 2 (n=10), and 3 (n=13) years after transplantation, and results were compared with results of seven nontransplanted controls undergoing diagnostic catheterization for atypical chest pain. No transplant patient had significant (>50%) epicardial coronary stenoses. All controls had angiographically normal epicardial coronary arteries. Controls were studied concurrently with the transplant cohort; their responses have been presented previously.6 Clinical and laboratory information collected from transplanted patients included age of donor heart, age of transplant recipient, sex, years after transplantation, pretransplant history of ischemic heart disease, biopsy-documented active rejection at time of study, total episodes of rejection since transplantation, mean total cholesterol level since transplantation, mean trough cyclosporine level, and history of hypertension (see "Definition of Clinical Factors" above).

Protocol

Written informed consent was obtained from all patients before the diagnostic catheterization in accordance with guidelines established by the Committee for the Protection of Human Subjects at Brigham and Women’s Hospital. Vasoactive medications were discontinued 18-24 hours before catheterization.

Diagnostic right and left heart catheterization and coronary angiography were performed by a standard percutaneous femoral approach. After completion of the diagnostic catheterization, an additional 5,000 units heparin was given intravenously, and an 8F guiding catheter was positioned in the ostium of the left coronary artery. A 20-MHz pulsed Doppler crystal mounted on the tip of a 3F infusion catheter (Millar Instruments Inc., Houston, Tex.) was advanced through the guiding catheter into the proximal segment of the left anterior descending coronary artery. The use of this device to assess intracoronary blood flow velocity has been described in detail.9 The Doppler catheter was connected to a photographic multichannel oscillographic recorder (Electronics for Medicine VR16, Pleasantville, N.Y.) to display phasic and mean velocity waveforms. Before the experimental protocol was begun, the position of the Doppler flow-velocity catheter and the range gate control were adjusted to optimize the audio flow-velocity signal and the phasic flow-velocity waveform. The Doppler catheter position and the range gate control were not changed thereafter.

Serial 2-minute intracoronary infusions of acetylsalicylic acid and adenosine were administered at a rate of 0.8 ml/min via the central lumen of the Doppler catheter in the following sequence: control (5% dextrose with heparin U/ml), graded concentrations of acetylsalicylic acid (to achieve estimated final blood concentrations of 10^-8, 10^-7, and 10^-6 M based on assumed left anterior descending coronary artery coronary blood flow of 80 ml/min), repeat control (0.9% sodium chloride with heparin U/ml), and graded concentrations of adenosine (Sigma Chemical Co., St. Louis, Mo.) (to achieve estimated final blood concentrations of 10^-6, 10^-5, and 10^-4 M). With the increase in blood flow observed at higher doses, the actual concentrations were proportionately lower. Just before the end of each infusion, coronary arteriography was performed in biplane orthogonal views with the use of a power injection of nonionic contrast medium (Omnipaque, Winthrop-Breon, New York). Throughout each infusion, the heart rate, arterial pressure, coronary flow velocity, and ECG (lead I) were monitored continuously, and all measurements were recorded in steady-state conditions.

Quantitative Coronary Angiography

Quantitative coronary angiography was performed by a previously validated technique.10 Nonionic contrast medium was injected into the left coronary artery at the rate of 5-10 ml/sec to a total of 7-12 ml with the use of a power injector (Medrad, Pittsburgh, Pa.) to optimize the quality and reproducibility of the injections.11 A biplane system (Polydiagnost-C, Philips Medical Systems, Inc., Shelton, Conn.) with two image intensifiers was used, and the left anterior descending coronary artery was positioned in the center of each field of view and at a single position in space (isocenter).
Analysis of Arterial Dimensions

Quantitative angiography of the epicardial coronary artery was performed for two reasons: 1) to determine cross-sectional area near the Doppler tip to convert flow velocity to an estimate of coronary arterial flow (an arterial segment 2–4 mm distal to the Doppler tip was selected for quantitative analysis in all patients), and 2) to exclude coronary artery flow limitation due to epicardial coronary artery constriction in response to acetylcholine (flow limitation defined as >50%-diameter constriction in the most constricted segment). Quantitative angiographic analysis was similar to that described in previous studies. Four digitized cine frames for each infusion were summed and averaged along the segment profile to give a mean diameter and SD at each point. A single mean and a pooled SD for the segment (at each infusion) were obtained by averaging each of these measures along the segment profile. Suitable segments were required to have a mean SD <5% of the mean diameter.

Estimates of CBF Changes

Relative changes in CBF were measured by multiplying changes in mean CBF velocity (as measured directly by the Doppler catheter) by changes in estimated vessel cross-sectional area (determined from the change in vessel diameter 2–4 mm distal to the catheter tip relative to control).

Statistical Analysis

Variations in hemodynamic parameters during the study and differences in dose–response curves to acetylcholine and adenosine were evaluated using repeated-measures ANOVA. Analysis of the association of clinical factors (transplant recipient age, total episodes of rejection, age of donor heart, sex, pretransplant history of ischemic heart disease, history of hypertension) and laboratory factors (active rejection, mean cholesterol level, mean cyclosporine level, arterial pressure, and heart rate) with the responses to drug infusions was performed using stepwise linear regression and repeated-measures ANOVA. Because results were similar when performing the statistical analysis with and without serially studied patients, the reported results include all studies. Statistical significance was assumed if the null hypothesis (two-tailed where appropriate) could be rejected at the p=0.05 level. All data are expressed as mean±SEM.

Results

Baseline Clinical and Hemodynamic Characteristics

Forty studies were performed in 29 transplanted patients (mean age, 42±2 years; age range, 20–63 years; four were female). Seventeen studies were done in patients 1 year after transplantation (Yr 1 TX), 10 were performed 2 years after transplantation (Yr 2 TX), and 13 were performed 3 years after transplantation (Yr 3 TX). Eleven patients were studied twice (five at years 1 and 2, five at years 2 and 3, and one at years 1 and 3). Ten had a pretransplant history of ischemic heart disease. Only two had no history of hypertension, and only four had active rejection on biopsy at time of study. Mean number of rejection episodes since transplantation was 4.2±0.5 (range, 1–10). Mean cholesterol levels ranged from 148 to 333 mg/dl (only two patients had mean total cholesterol levels <200 mg/dl) with a group mean cholesterol level of 258±6 mg/dl. A total cholesterol level was available for 100% of the months after transplantation. Mean trough cyclosporine levels ranged from 99 to 261 ng/ml with a group mean cyclosporine level of 165±7 ng/ml. A trough cyclosporine level was available for 85% of the months after transplantation. Mean age of the donor heart was 24±1 years (range, 16–41 years).

Mean age of control patients was 40±2 years (range, 31–48 years); one was female. Clinical, echocardiographic, and hemodynamic data for these patients have been previously reported. These patients had atypical chest pain, normal wall thickness and ventricular function on echocardiogram, and angiographically normal coronary arteries at the time of catheterization.

In addition to the 40 studies in 29 patients, we were unable to assess microvascular responses to acetylcholine in 13 transplant patients because of flow-limiting epicardial constriction to acetylcholine. Clinical and laboratory evaluations revealed these patients to be similar to our study population. This group had a mean age of 39±4 years (age range, 16–60 years); five were female. Two had a pretransplant history of ischemic heart disease. Twelve had a history of hypertension. Two had rejection on biopsy obtained at the time of catheterization. Mean total episodes of rejection since transplantation were 3.9±0.5. Group mean total cholesterol level was 252±12 mg/dl, and group mean trough cyclosporine level was 160±12 ng/ml. Mean age of the donor heart was 22±2 years (range, 16–35 years). Five were Yr 1 TX, three were Yr 2 TX, and five were Yr 3 TX. One was a year-3 study of a patient who had been studied in years 1 and 2 and subsequently developed significant epicardial arteriosclerosis. One was a year-2 study of a patient who had been studied in year 1 and subsequently developed significant epicardial arteriosclerosis.

Echocardiographic evaluation of transplanted patients (Table 1) revealed left ventricular posterior wall thickness of 1.11±0.03 cm, left ventricular end-diastolic dimension of 4.6±0.1 cm, and left ventricular end-systolic dimension of 2.9±0.1 cm. Control patients had left ventricular posterior wall thickness of 0.99±0.01 cm, left ventricular end-diastolic dimension of 5.0±0.1 cm, and left ventricular end-systolic dimension of 3.1±0.2 cm.

Mean baseline heart rate for transplant patients was 83±2 versus 63±3 beats per minute for control patients (p<0.05). Mean baseline systemic arterial pressure was 113±2 mm Hg for transplant patients and 89±6 mm Hg for control patients (p<0.05). Left ventricular end-diastolic pressure was 13±1 mm Hg for transplanted patients and 12±2 mm Hg for control patients (p=NS). Left ventricular ejection fraction was 0.67±0.01 for transplanted patients (by echocardiography) and 0.76±0.04 for control patients (by ventriculography in four and echocardiography in three) (p<0.05).

Systemic Hemodynamic Responses to Drug Infusions

Heart rate (beats per minute) did not change significantly with acetylcholine or adenosine in either group (transplant: baseline, 83±2; peak acetylcholine, 84±2; recontrol, 84±2; peak adenosine, 84±2; control: base-
line, 63±3; peak acetylcholine, 62±3; recontrol, 62±4; peak adenosine, 59±4) (all p=NS).

Mean systemic arterial pressure (mm Hg) did not change significantly with acetylcholine or adenosine in the control group (control: baseline, 89±6; peak acetylcholine, 89±8; recontrol, 89±7; peak adenosine, 96±8) (all p=NS) but decreased slightly with adenosine in the transplant group (transplant: baseline, 113±2; peak acetylcholine, 114±2; recontrol, 113±2; peak adenosine, 109±3; p<0.05 for peak adenosine versus recontrol).

Epicardial Coronary Responses to Acetylcholine and Adenosine

The distal large coronary artery responses to acetylcholine were analyzed only in patients with distal epicardial coronary artery segments that showed potentially flow-limiting constriction in response to acetylcholine infusion. Thirteen patients had >50% constriction of their epicardial coronary artery distally and these patients were excluded. Of the remaining 40 studies of 29 patients, eight studies demonstrated constriction to acetylcholine requiring analysis of the distal segment. The mean degree of constriction in this group of patients was −11.4±2.6%.

Epacrdial coronary artery diameter changes at the Doppler tip were analyzed in all 40 studies to convert flow velocity to relative changes in CBF. Mean overall change in epicardial diameter in response to acetylcholine at the Doppler tip was −0.2±1.7% (1.5±3.1% in Yr 1 TX patients, 1.8±3.7% in Yr 2 TX patients, and −4.0±2.3% in Yr 3 TX patients). Mean overall change in epicardial diameter in response to adenosine at the Doppler tip was a dilation of 15.4±2.4% (15.9±3.5% in Yr 1 TX patients, 13.5±7.2% in Yr 2 TX patients, and 16.0±3.4% in Yr 3 TX patients).

CBF Responses to Acetylcholine

CBF responses to acetylcholine were similar in controls, Yr 1 TX, and Yr 2 TX but diminished in Yr 3 TX (Figure 1). Controls increased CBF 32±15%, 128±40%, and 232±40% in response to serial doses of acetylcholine (10⁻⁸ M, 10⁻⁷ M, and 10⁻⁶ M, respectively). Yr 1 TX increased CBF 3±8%, 61±19%, and 200±41% (p=NS versus controls). Yr 2 TX increased CBF 33±12%, 110±42%, and 201±54% (p=NS versus controls). However, Yr 3 TX increased CBF only 12±9%, 39±13%, and 100±39% in response to serial doses of acetylcholine (p=0.03 versus controls by repeated-measures ANOVA).
CBF Responses to Adenosine

CBF responses to serial doses of adenosine demonstrated a trend toward impairment over time but did not achieve statistically significant differences (Figure 2). CBF responses to adenosine ($10^{-6}$ to $10^{-4}$ M) were $163\pm37\%$, $293\pm39\%$, and $438\pm54\%$ in controls; $297\pm102\%$, $394\pm149\%$, and $430\pm61\%$ in Yr 1 TX; $222\pm62\%$, $426\pm67\%$, and $384\pm61\%$ in Yr 2 TX; and $164\pm48\%$, $254\pm39\%$, and $329\pm35\%$ in Yr 3 TX (all $p=NS$ by repeated-measures ANOVA).

Endothelium-Dependent CBF Responses

To examine the endothelium-dependent component of vasodilation, the flow responses to acetylcholine in each patient were normalized by the maximal flow response to adenosine. The data (Figure 3) in those patients receiving both acetylcholine and adenosine show that the endothelium-dependent flow response deteriorated over time and was significantly impaired by the third year after transplantation. Control ($11\pm6\%$, $35\pm12\%$, and $57\pm9\%$ representing acetylcholine $10^{-8}$ M/peak adenosine, acetylcholine $10^{-7}$ M/peak adenosine, and acetylcholine $10^{-6}$ M/peak adenosine, respectively) and Yr 1 TX ($3\pm3\%$, $17\pm6\%$, and $56\pm10\%$) ($p=NS$) had similar measures of endothelium-dependent vasodilation. Yr 2 TX had a diminishing response ($4\pm1\%$, $18\pm4\%$, and $47\pm12\%$) and Yr 3 TX ($3\pm3\%$, $8\pm4\%$, and $29\pm9\%$) had a significantly lower measure.
of endothelium-dependent dilation ($p=0.03$ for control versus Yr 3 TX by repeated-measures ANOVA).

Of the patients undergoing serial studies, there was a trend toward a decreased endothelium-dependent flow response (peak acetylcholine/peak adenosine response, $72\pm21\%$ to $36\pm14\%$ in patients studied in years 1 and 2, and $41\pm20\%$ to $32\pm36\%$ in patients studied in years 2 and 3) (Figure 4), although these differences did not reach statistical significance.

**Predictors of the Endothelium-Dependent Flow Response**

By repeated-measures ANCOVA and stepwise linear regression, mean trough cyclosporine level ($r=0.67$, $p=0.004$) and transplant recipient age ($r=0.51$, $p=0.004$) were the only correlates of the endothelium-dependent flow response (peak acetylcholine response/peak adenosine response) among all factors analyzed (donor age, sex, pretransplant history of ischemic heart disease, years after transplantation, active rejection, total episodes of rejection, history of hypertension, arterial pressure at time of study, and mean cholesterol level). A preserved endothelium-dependent response correlated with increased cyclosporine levels and increased transplant recipient age. After exclusion of active rejection on biopsy, history of hypertension and hypercholesterolemia, cyclosporine level, and transplant recipient age continued to be the only significant predictors of the endothelium-dependent response. Univariately, the correlation of cyclosporine to the endothelium-dependent flow response was similar from year to year, indicating that this association was not dependent on years after transplantation. Although only $85\%$ of the months had cyclosporine levels available for analysis, the differences in cyclosporine levels between years and the correlation of cyclosporine levels with the microvascular endothelial response cannot be explained by the percentage of data missing ($p=0.24$).

The slope of each patient’s endothelium-dependent flow response was calculated to allow graphic representation of the relation of cyclosporine to microvascular endothelial function (Figure 5). A more positive slope (increasing flow with increasing acetylcholine dose) reflects microvascular dilation. Again, a preserved endothelium-dependent response correlated with increased cyclosporine levels ($r=0.52$, $p<0.05$).

**Pathological Evaluation**

In one patient who died as a result of ischemic left ventricular dysfunction 10 months after his year 3 study (3 years and 10 months after transplant), pathological evaluation revealed marked intimal proliferation with near-obliteration of the vessel lumen consistent with graft arteriosclerosis (Figure 6). His microvascular endothelium-dependent vasodilator response was impaired in his year 3 study (50% increase in CBF in response to acetylcholine $10^{-8}$ M; only 24% of his flow response was attributable to endothelium-dependent vasodilation).

**Discussion**

In a cohort of cardiac transplant patients, we assessed the functional integrity of the coronary microvasculature. By recording CBF at constant pressure, we measured changes of small-vessel resistance—microvascular vasomotor response. The responses to the endothelium-dependent vasodilator acetylcholine demonstrated a loss of normal microvascular endothelium-dependent vasodilation over time. A trend suggesting progressive impairment of the vasodilator response to adenosine was also observed. However, in normalizing the acetylcholine response by the adenosine response in each case, we found the endothelium-dependent vaso-
The endothelium is an important regulator of vascular tone. The release of endothelium-derived relaxing factor is a mode of action of a variety of vasodilators, including acetylcholine, ADP, ATP, histamine, bradykinin, substance P, thrombin, and calcitonin gene-related peptide. Studies in the large (conduit) coronary arteries have shown that diseases such as atherosclerosis and transplant-associated arteriosclerosis damage the endothelium and impair the normal relaxation response to acetylcholine. Fish et al demonstrated that the ability of epicardial coronary arteries to dilate to acetylcholine is impaired in the majority of patients by 1 year after transplantation, suggesting that endothelial damage occurs early after transplantation.

Recent studies have demonstrated that endothelium-mediated vasomotion not only is important in control of large-vessel tone but also plays an important role in the control of small (resistance) vessels. Collagenase digestion of endothelium or inactivation of endothelium-derived relaxing factor by hemoglobin markedly dilator function of the microvasculature to be impaired beyond the possible effect of underlying vasomotor incompetence. These findings may reflect the progressive nature of graft arteriosclerosis.

The endothelium is an important regulator of vascular tone. The release of endothelium-derived relaxing factor is a mode of action of a variety of vasodilators, including acetylcholine, ADP, ATP, histamine, bradykinin, substance P, thrombin, and calcitonin gene-related peptide. Studies in the large (conduit) coronary arteries have shown that diseases such as atherosclerosis and transplant-associated arteriosclerosis damage the endothelium and impair the normal relaxation response to acetylcholine. Fish et al demonstrated that the ability of epicardial coronary arteries to dilate to acetylcholine is impaired in the majority of patients by 1 year after transplantation, suggesting that endothelial damage occurs early after transplantation.

Recent studies have demonstrated that endothelium-mediated vasomotion not only is important in control of large-vessel tone but also plays an important role in the control of small (resistance) vessels. Collagenase digestion of endothelium or inactivation of endothelium-derived relaxing factor by hemoglobin markedly dilator function of the microvasculature to be impaired beyond the possible effect of underlying vasomotor incompetence. These findings may reflect the progressive nature of graft arteriosclerosis.

The endothelium is an important regulator of vascular tone. The release of endothelium-derived relaxing factor is a mode of action of a variety of vasodilators, including acetylcholine, ADP, ATP, histamine, bradykinin, substance P, thrombin, and calcitonin gene-related peptide. Studies in the large (conduit) coronary arteries have shown that diseases such as atherosclerosis and transplant-associated arteriosclerosis damage the endothelium and impair the normal relaxation response to acetylcholine. Fish et al demonstrated that the ability of epicardial coronary arteries to dilate to acetylcholine is impaired in the majority of patients by 1 year after transplantation, suggesting that endothelial damage occurs early after transplantation.

Recent studies have demonstrated that endothelium-mediated vasomotion not only is important in control of large-vessel tone but also plays an important role in the control of small (resistance) vessels. Collagenase digestion of endothelium or inactivation of endothelium-derived relaxing factor by hemoglobin markedly dilator function of the microvasculature to be impaired beyond the possible effect of underlying vasomotor incompetence. These findings may reflect the progressive nature of graft arteriosclerosis.
inhibited the microvascular dilator response to acetylcholine in rabbits\textsuperscript{16} and rats.\textsuperscript{17} In this and previous studies, we have demonstrated that normal humans exhibit dose-dependent dilatation of the coronary resistance vessels in response to acetylcholine, a function that is likely to be endothelium dependent.\textsuperscript{9}

Graft arteriosclerosis is a progressive, diffuse, concentric process that begins early after transplantation and can involve the entire coronary tree.\textsuperscript{1,2,19-23} The pathogenetic mechanism probably is related to immune-mediated damage of the vascular endothelium, although it remains uncertain whether graft arteriosclerosis is a cell-mediated process, a humoral process, or a combination of the two. Theories regarding the role of cytotoxic B cell antibodies,\textsuperscript{22} antiendothelial cell antibodies,\textsuperscript{23,24} and upregulation of HLA class II major histocompatibility complex antigens of the vascular endothelium (with a subsequent increase in leukocyte binding and proliferation of inflammatory lymphokines)\textsuperscript{25} have found support. Recently, Salomon et al\textsuperscript{26} found increased class II major histocompatibility complex expression associated with increased T lymphocytes and HLA-DR+ macrophages on the endothelium of human cardiac allografts, suggesting that graft arteriosclerosis is a chronic immune reaction to activated graft endothelial cells.

In this study, we found a positive correlation of cyclosporine levels with microvascular endothelium-dependent vasodilation. The effect of cyclosporine on endothelial cell function is controversial. Bossaller et al,\textsuperscript{27} in the nontransplanted rat aorta, found that cyclosporine acutely impairs endothelium-dependent vasodilation and decreases prostacyclin production. Others have seen no effect of cyclosporine on the ability of the endothelium to produce and release endothelium-derived relaxing factor.\textsuperscript{28} To our knowledge, no study has evaluated the effect of cyclosporine on endothelium-dependent vasodilation and endothelium-derived relaxing factor production in vessels damaged by graft arteriosclerosis. We speculate that our observed association of increased cyclosporine levels with preserved microvascular endothelial function may be a direct result of cyclosporine's suppression of the immune process on the endothelial surface of the graft. It is equally possible that this is an association of two pathophysiologically unrelated factors. Further investigation of the effects of cyclosporine on endothelial function in the transplant model is warranted.

The role of cyclosporine as an inhibitor or promoter of graft arteriosclerosis is also controversial. Zusman et al,\textsuperscript{29} in a study of patients 1–4 years after transplantation, found that 72\% of cyclosporine-treated patients were free of graft arteriosclerosis, whereas only 61\% of conventionally treated (azathioprine and prednisone) patients had no graft arteriosclerosis. Lurie et al,\textsuperscript{20} in a rat heart transplant model, found that cyclosporine effectively prevented the development of graft arteriosclerosis. However, in rat aortic allografts, cyclosporine treatment produces "endothelialitis" and accelerated arteriosclerosis.\textsuperscript{30}

Transplant recipient age also was positively correlated with the microvascular endothelial vasodilator response. Although this association is weaker than that of cyclosporine with endothelium-mediated vasodilation, one potential explanation for this finding could relate to changes in the immune response with age. We can only speculate that older transplant recipients with less immunocompetence would suffer less graft microvascular immune-mediated damage and consequently have more preserved vasodilator responses to acetylcholine. We found no other clinical or laboratory factors that correlated with the microvascular endothelial vasodilator response.

\section*{Study Limitations}

Studies in an additional 13 patients could not be completed due to flow-limiting large-vessel constriction in response to acetylcholine; comparison of their clinical variables with those of the studied population did not show evident bias represented by their exclusion.

Because few patients had active rejection or diabetes mellitus but the majority had elevated cholesterol levels and hypertension, our analysis was necessarily less sensitive to the correlation of these factors with the microvascular endothelial vasodilator response. We are continuing to examine these correlates in extended follow-up of our growing cohort of transplant patients.

This study does not address mechanisms of endothelial dysfunction in the transplant population. The abnormality we describe may not be due solely to impaired endothelium-mediated vasodilation. Endothelium-derived relaxing factor release, free radical inactivation of endothelium-derived relaxing factor, or one of many other potential mechanisms observed in experimental models of disease may account for this abnormality.\textsuperscript{31-33} In addition, we cannot be sure whether these altered responses to acetylcholine represent altered maximal responses or altered vessel wall sensitivity, but either case is consistent with endothelial dysfunction.

In summary, we have demonstrated that microvascular endothelium-dependent vasodilation diminishes over time in cardiac transplant patients. This may reflect both primary deterioration of endothelial function and underlying vasomotor insensitivity as graft arteriosclerosis progresses. Loss of endothelium-mediated control of CBF may contribute to progressive ischemia and myocardial damage in the transplanted heart. Possibly, other functions of the endothelium (e.g., maintenance of an antithrombotic surface, growth factor production) may also be altered by graft arteriosclerosis, thereby contributing further to the process.

Considered together, our results raise expectations that therapies directed toward the protection of the microvessels, the endothelium in particular, from immune injury may beneficially alter the process of microvascular transplant arteriosclerosis. This process may be monitored by serial measurement of endothelium-dependent vasodilation, which in the future may provide a means of detecting clinically important transplant arteriosclerosis at an early stage. Improved understanding of microvascular endothelium-dependent function and the immune mechanisms that affect it may permit more specific and successful therapies for protection of the transplanted heart from ischemic damage and for improved long-term survival.

\section*{Acknowledgments}

We acknowledge the assistance of the technical staff of the Brigham and Women's Cardiac Catheterization Laboratory for their excellent help in performing these studies, and
George Cotsonis, MS, of the Emory University School of Public Health Biostatistics Consulting Center for his generous assistance with the statistical analysis.

References
Loss of the coronary microvascular response to acetylcholine in cardiac transplant patients.
C B Treasure, J A Vita, P Ganz, T J Ryan, Jr, F J Schoen, V I Vekshtein, A C Yeung, G H Mudge, R W Alexander and A P Selwyn

Circulation. 1992;86:1156-1164
doi: 10.1161/01.CIR.86.4.1156
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1992 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/86/4/1156

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/