Development of coronary artery disease in cardiac transplant patients receiving immunosuppressive therapy with cyclosporine and prednisone

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ABSTRACT  Coronary artery disease (CAD) has been shown in previous uncontrolled studies to be a limiting factor to long-term survival in patients undergoing cardiac transplantation and who were taking conventional immunosuppressants. To study the development of CAD after cardiac transplantation in patients taking the newer immunosuppressive agent cyclosporine, we prospectively performed yearly coronary arteriography on all eligible transplantation patients (first year, 57 patients; second year, 30 patients; third year, 14 patients). The prevalence of CAD by life table analysis was 18% at 1 year, 27% at 2 years, and 44% at 3 years. The occurrence of two or more major rejection episodes was associated (p < .005) with the development of CAD. In two patients who died of CAD, coronary artery histology revealed subintimal inflammatory cellular infiltration in some lesions. These data demonstrate that the prevalence of CAD rises progressively over time and immunologic factors may be important in its development.


ALTHOUGH SHORT-TERM (<1 year) survival after cardiac transplantation primarily relates to infectious complications, tissue rejection, and early (<2 weeks) graft failure, a major impediment to long-term survival may be the development of obstructive coronary artery disease (CAD) in the allograft.1

In the earliest clinical series on cardiac transplantation, almost all late survivors whose immunosuppression consisted of azathioprine and prednisone developed this complication.1, 2 It has been suggested that CAD in this setting is a manifestation of chronic graft rejection.3–7

In most transplant centers, cyclosporine has become the immunosuppressive drug of choice. It appears to be highly effective in maintaining adequate immunosuppression, allowing a decrease in chronic steroid dose. It may be that this agent will provide better protection against chronic rejection than previous immunosuppressive regimens, which in turn may decrease the severity of CAD. On the other hand, cyclosporine therapy after cardiac transplantation is almost universally associated with moderate-to-severe hypertension, which may in itself represent a risk factor for CAD in this setting.

To date, no study has prospectively examined by coronary arteriography the development of CAD in the posttransplant population on cyclosporine. This study was designed to examine the prevalence of CAD after cardiac transplantation and the factors associated with its development in patients treated with cyclosporine and prednisone over the first 3 years after surgery.

Materials and methods

Patient population. All adult orthotopic cardiac transplant recipients who were on long-term cyclosporine and prednisone therapy and who had at least one annual cardiac catheterization from June 1982 through February 21, 1986 were included in this study. Ninety-eight percent of eligible patients were actually enrolled in this study. Fifty-seven patients underwent at least one annual catheterization. Of these, 30 underwent a second annual study and 14 had a third annual procedure. The characteristics of the patient population at each catheterization time point are listed in table 1. Ischemic and idiopathic cardiomyopathy constituted the predominant cause of pretransplantation heart disease (table 2). The mean donor age was 23 ± 5 years.

Methods

Definitions

Major rejection episode. This event was defined as histologic evidence of rejection (grades 3 to 4,Billingham classification5) coupled with the clinical decision to treat with...
rabbit antithymocyte globulin and/or high-dose steroids. Consecutive biopsy samples demonstrating severe rejection were considered part of the same rejection episode.

**Coronary Artery Disease.** Coronary arteriography was performed at yearly intervals by the Judkins or Sones technique. Coronary arteriography was reviewed by three of the authors (B. F. U., P. S. R., S. M.) separately, and a consensus was reached regarding the presence of CAD and degree of luminal narrowing. CAD was defined as any decrease in luminal diameter ranging from luminal irregularities (<10%) to "significant" (≥50%) luminal obstruction.

**Antiplatelet agents.** Aspirin (600 mg/day) (n = 20), dipyridamole (50 mg tid) (n = 14), or both (n = 8) was given as antiplatelet therapy. These agents were not given in a prospective randomized manner.

**Blood samples.** Postoperative cholesterol and triglyceride levels were obtained at varying intervals over the first year, frequently by the referring physician. These samples were not prospectively obtained.

**Specific methods.** A direct immunofluorescence procedure was used to detect immunoglobulin and complement in capillaries and small vessels in the myocardial biopsy samples. The samples were frozen in embedding medium within 30 min of collection. Goat antihuman immunoglobulin (Ig)G, IgA, IgM, and C3 conjugated to fluorescein were used.9 The serum samples were applied to cryostat cut sections of tissue. As a control, goat antihuman albumin conjugated to fluorescein was used. The tissue was viewed under a Leitz epifluorescence microscope with the use of a 50 watt xenon lamp. Reactions were considered positive or negative. A negative reaction had no labeling with antisera to Ig or complement. A positive reaction was recorded when the antialbumin serum had a negative reaction but either the antimforcin IgG or complement serum was positive.

As part of the pretransplant cross matching, cytotoxic antibody to donor T and B lymphocytes was determined with the use of serum from the recipient. A two-color immunofluorescence procedure using ethidium bromide to detect dead cells was used.10 If a recipient had cytotoxic antibody to donor T lymphocytes, the transplant was not performed. However, antibody to donor B lymphocytes did not preclude transplantation. HLA antigens were determined by the standard Amos procedure with the use of locally prepared trays.11

**Statistical methods.** Data are presented as the mean ± SD.

### TABLE 1
**Characteristics of the posttransplantation population undergoing coronary arteriography**

<table>
<thead>
<tr>
<th>Catheterization time point (year)</th>
<th>Number of patients</th>
<th>Patient age (years)</th>
<th>Sex (male/female)</th>
<th>Cyclosporine dose (mg/kg/day)</th>
<th>Prednisone dose (mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>57</td>
<td>43 ± 10</td>
<td>49:8</td>
<td>5.4 ± 3</td>
<td>22 ± 7</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>41 ± 9</td>
<td>24:6</td>
<td>4.6 ± 2</td>
<td>17 ± 6</td>
</tr>
<tr>
<td>3</td>
<td>14</td>
<td>43 ± 9</td>
<td>13:1</td>
<td>4.0 ± 3</td>
<td>12 ± 6</td>
</tr>
</tbody>
</table>

To compare differences in groups, the chi-square method or unpaired t test was used when appropriate. Life table analysis was used to determine the risk of CAD at each time point.

### Results

Life table analysis showed a risk of developing CAD of 18% at 1 year, 27% at 2 years, and 44% at 3 years (figure 1). The actual prevalence of CAD in the population was quite similar, showing a 19% prevalence at 1 year, 20% at 2 years, and 43% at 3 years. It should be noted that only two patients in our entire series had significant CAD (i.e., >50% luminal narrowing) and these lesions were 50% mid left anterior descending luminal narrowing observed at the third year study in one patient, and a 90% proximal circumflex lesion at the 2 year time point in another. However, CAD was progressive (figure 2). Of the six patients who had CAD at 2 years, two developed it de novo after the first year. The first patient developed diffuse luminal irregularities of the obtuse marginal branch of the left circumflex artery and the second patient developed luminal irregularities of the acute marginal branch of the right coronary artery at the second year after normal coronary.

### FIGURE 1
Life table analysis showing the risk of developing CAD over the first 3 years after cardiac transplantation (TX).
The third patient had luminal irregularities of the mid left anterior descending artery and the fourth patient had luminal irregularities of the distal left circumflex artery, neither of which progressed from the first to the second year study.

Of the six patients with CAD at the third year, four developed it de novo after the second year study. The first patient developed luminal irregularities of the mid left anterior descending artery, the second patient developed a 30% lesion of the mid left anterior descending artery and diffuse luminal irregularities of the distal right coronary artery and posterior descending artery, the third patient developed a 40% mid left descending artery lesion and luminal irregularities of the distal right coronary and posterior descending arteries, and the fourth patient developed two discrete lesions of the mid left anterior descending artery (40% and 50%). In addition, this patient also developed luminal irregularities of the proximal left circumflex artery and diffuse luminal irregularity of the entire right coronary artery. All of the above four patients had normal coronary arteriograms at the first and second year studies.

The remaining two patients with CAD at the second year study showed progression of the number of vessels involved at the third year study. In one patient, luminal irregularities of the obtuse marginal branch of the left circumflex artery progressed to include luminal irregularities of the major diagonal branch of the left anterior descending artery. In the other patient, luminal irregularities of the acute marginal branch of the right coronary artery progressed to include luminal irregularities of the proximal left anterior descending artery and proximal left circumflex artery, and the entire right coronary artery.

Overall, the extent of CAD increased with time. Single-vessel disease was more common in the first and second year studies, whereas multivessel disease was more prevalent at the third year time point (table 3).

The presence of two or more rejection episodes was significantly associated (p < .005 at 1 year, p < .01 at 2 years) with an increased prevalence of CAD (figure 3). Sixty-four percent of patients with CAD at 1 year and 83% of those with CAD at 2 years had recurrent major rejection episodes, whereas only 17% and 25% of patients without CAD at 1 and 2 years had major rejection episodes. There were fewer patients on antiplatelet therapy (either aspirin or dipyridamole alone or in combination) in the group with CAD (25% at 1 year, 33% at 2 years) than in the group without CAD (50% at 1 year, 73% at 2 years), but this difference did not reach statistical significance. Other factors not significantly associated with the development of CAD included abnormal renal function (creatinine > 2 mg%), donor age, posttransplantation smoking, post-

### TABLE 3

<table>
<thead>
<tr>
<th>Extent of coronary artery disease</th>
<th>Year after transplant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First (n = 11)</td>
</tr>
<tr>
<td>Single-vessel disease</td>
<td>64%</td>
</tr>
<tr>
<td>Double-vessel disease</td>
<td>18%</td>
</tr>
<tr>
<td>Triple-vessel disease</td>
<td>18%</td>
</tr>
</tbody>
</table>
operative serum cholesterol level, presence of pretransplant cytotoxic B cell antibody, and the presence of pretransplant ischemic heart disease.

In a subgroup of patients who underwent HLA tissue typing (n = 43), there was a higher prevalence of CAD in patients with two or more HLA-A and B mismatches (100% vs 0%), but this did not reach statistical significance because 39 of 43 patients (91%) had two or more mismatches. All the patients with CAD had at least 1 HLA-DR mismatch, but again since 86% had at least one mismatch this difference did not reach statistical significance. It should be noted that no patient had total histocompatibility in both the HLA-A/B and DR series. In a smaller subgroup (n = 16), the presence of Ig (either IgG, IgA, or IgM) and complement in the smaller intramyocardial coronary arteries in endomyocardial samples taken during endomyocardial biopsy did not correlate with the presence of CAD at 1 year.

Since the reinstitution of our cardiac transplant program in May 1981, through February 21, 1986, there have been 53 deaths among adult orthotopic transplant recipients (figure 4). Forty-six deaths occurred in the first 12 months after transplantation. The two leading causes of death included infection (34%) and rejection (33%). The third leading cause of death (24%) was early (<14 days) graft failure presumed to be either from hyperacute rejection, early donor right heart failure (usually in patients with preoperatively increased pulmonary vascular resistance), or from inadequate donor preservation. In most cases, the exact cause of graft failure was not possible to discern. There were no deaths due to CAD. Of the seven patients who died 13 to 36 months after transplantation, autopsy results were available for six (table 4). None of these six patients had CAD by coronary arteriography, but four showed some degree of CAD at autopsy. CAD was considered the cause of death in two, secondary to silent myocardial infarction and sudden death. In these two patients, atherosclerotic lesions consisted of fibrointimal hyperplasia, with others showing inflammatory cellular infiltrate (figure 5).

**Discussion**

This study demonstrates the development of progressive obstructive CAD over the first 3 years after cardiac transplantation in patients treated with cyclosporine and prednisone immunosuppression. Available data from the medical literature do not allow for a true comparison of the relative prevalence of CAD with the present immunosuppressive regimen as opposed to azathioprine and prednisone. In the earliest patient series from Stanford (1968–1969), CAD developed in all 3 year survivors (nine of nine patients).1 In a second group of Stanford patients (1970–1975) prevalence of CAD at 3 years was 17% (seven of 44 patients); how-

**TABLE 4**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Time between cath and death (months)</th>
<th>Cath</th>
<th>Autopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>0</td>
<td>99% proximal LAD, plaques in LCX and RCA</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>0</td>
<td>80% proximal LAD, 75% proximal LCX, plaques in RCA</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>0</td>
<td>20% RCA, 15% LCX with occluded branch, 25% LAD lateral wall MI (2–4 days old)</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>0</td>
<td>20% proximal LAD, plaques in LCX and RCA</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

LAD = left anterior descending artery; LCX = left circumflex artery; RCA = right coronary artery; MI = myocardial infarction.
FIGURE 5. Low-power (6 x, A), and high-power (120 x, B) views of the left anterior descending artery and low-power (6 x, C) and high-power (120 x, D) views of the circumflex artery are shown for patient 3. Note that the histologic characteristics of the anterior descending are those of primary fibromuscular hyperplasia (B), while the circumflex artery contains a cellular infiltrate (D). Note also that a side branch of the circumflex (C) is totally occluded by thrombus.

ever, only 67% of eligible patients were included. A third Stanford series, published in 1974, showed no patients (none of five) with CAD at 3 years; in this presentation, selection criteria for catheterization were not clear, and considering the small number of cases, it is likely a selection bias existed in this series as well. Finally, in a more recent study from Stanford, in 85 1 year survivors, the incidence of CAD was 27%. Based on the presented material, it is not possible to ascertain at which point in time this incidence was present, or the percent of eligible patients actually undergoing catheterization at this time. The present study would thus appear to be the first published large prospective study to quantitate both the prevalence and severity of CAD over the first 3 years after cardiac transplantation, particularly in cyclosporine-treated patients.

The occurrence of two or more major rejection episodes was the only factor studied that was associated with the development of CAD. This association has previously been suggested in a smaller series of patients treated with azathioprine and prednisone. Hess et al. have demonstrated that the posttransplantation presence of a cytotoxic B cell antibody, which may be directed at an HLA-DR antigen on the vascular endothelium, showed an association with severe and early developing CAD. Since our measurement of the presence of a cytotoxic B antibody was limited to a preoperative sample, we can neither confirm nor refute the data presented by Hess et al. In the same study, serum cholesterol, particularly when combined with the presence of cytotoxic B cell antibody, was associated with CAD; in another study, cholesterol was not associated with CAD. In our study, we did not find an association. This negative finding must, however, be interpreted cautiously. In a recent preliminary study, it has been shown that cholesterol rises progressively over the first year after transplantation. Since serum cholesterol was measured at different times in different patients after transplantation in our study, the lack of association may be related to methodologic considerations rather than a true lack of association. The relative
unimportance of traditional risk factors (smoking, diabetes, hypertension) in the development of CAD do not negate their potential importance. It should be emphasized that hypertension was present to some degree in all patients. On the other hand, the presence of smoking (9%) and diabetes (14%) was rather low after transplantation.

There are several pieces of evidence that suggest that the obstructive coronary lesion in this setting may be immunologically mediated. In addition to the association of recurrent rejection episodes and CAD in this study, and the association of cytotoxic B cell antibodies in the study by Hess et al., animal studies have demonstrated that CAD can be provoked by immunologic means. In a rabbit preparation in which foreign protein was repeatedly injected and animals were fed a lipid-rich, cholesterol-poor diet, CAD developed. The major arterial histologic finding was intimal thickening similar to that seen in human atherosclerosis as well as in some of the CAD lesions seen in our series (figure 5, A and B). Recently, a single case has been reported of severe diffuse small-vessel and distal epicardial CAD with vascular IgM and complement (C3) deposits. Histologic examination revealed both intimal proliferative changes and “focal mononuclear cell infiltrates.”

In a subsequent study in which rabbits were injected with foreign protein and fed a cholesterol-rich diet for 80 days after protein challenges, the major histologic lesions were fibromuscular hyperplasia and lipid-laden (“foam”) cells. The authors noted that the lesions produced resembled human atherosclerosis; these lesions were similar to some of the CAD lesions seen in our series (figure 6). Conspicuously absent in this immunemediated animal preparation of atherosclerosis was the inflammatory cellular infiltrate seen in the present series. The same investigative group also evaluated CAD histology in murine heterotopic heart transplantation. They found that both of the above histologic lesions were present, but additionally a cellular inflammatory infiltrate comprised primarily of lymphocytes and plasma cells was seen in some lesions. This type of lesion is not characteristic of human atherosclerosis. It has been seen, however, in lesions in patients treated with azathioprine and prednisone immunosuppression, as well as in some of the lesions in this series (figure 5, D).

We propose that CAD in the posttransplant setting

**FIGURE 6.** A high-power view (120×) of a lesion in patient 1 demonstrating a predominance of lipid-laden (“foam”) cells.
is a manifestation of chronic tissue rejection and tissue response. Such a sequence of events may be akin to what is seen in the myocardium. In this setting, a stimulus or stimuli, as yet not characterized, provokes the cellular rejection reaction, which when treated either resolves without histologic sequelae or the development of fibrosis. We suggest the coronary artery tissue rejection also provokes, at least in some cases, a cellular response, which damages the endothelial barrier. Thus, a cellular infiltrate represents an early lesion. Cholesterol and circulating proteins, analogous to the foreign protein injected in the rabbit, enter subintonally, thus stimulating fibromuscular hyperplasia and foam cell production (later lesions). On the other hand, it may be that circulating Igs in some or all cases directed against the vascular endothelium provoke the immune response leading to CAD.

Further suggestive data were also gathered from our patient who had an active cellular infiltrate in the occluded circumflex artery branch adjacent to the fresh myocardial infarction while another part of the arterial tree had histlogic characteristics compatible with a noninflammatory stage (fibromuscular hyperplasia).

The results of this study have certain implications for the prevention of CAD after transplantation. If, indeed, CAD is a form of chronic rejection initiated by a lymphocytic reaction to vascular tissue, and its development is associated with recurrent rejection episodes seen in the myocardium, the major thrust of prevention will be to develop more effective immunosuppressive regimens and methods to diagnose rejection. If, on the other hand, circulating Igs appear to provoke vascular change and initiate the atherosclerotic process, then approaches other than inhibiting cell-based rejection will be required. The usefulness of platelet-inhibiting drugs in this setting still remains unclear. Although an earlier study by the Stanford group suggested that the use of warfarin and dipyridamole might decrease the incidence of CAD, this study had several methodologic problems and a controlled study in man has yet to be performed. The decrease in CAD described by the Stanford group was coincident with use of the endomyocardial biopsy technique for monitoring rejection, so the decreased incidence of CAD may have been due to improvements in immunologic monitoring. The uncontrolled observations on the use of antiplatelet agents in this study suggest a controlled trial may be worthwhile. Furthermore, an animal study has suggested cyclosporine and dipyridamole, but not cyclosporine alone, may prevent the development of posttransplant CAD.

The lack of arteriographic obstructive lesions in the patients undergoing autopsy and the presence of inflammatory lesions in some of these cases suggest that CAD may in certain cases develop rather rapidly. Coronary arteriography may miss this relatively rapid change. Additionally, coronary arteriography may underestimate the amount of diffuse concentric atherosclerosis.

Arteriographic findings demonstrating rather diffuse luminal changes suggest that revascularization procedures, either bypass surgery or coronary angioplasty, will be useful in only a small percentage of patients and that in patients with severe diffuse arteriosclerosis, retransplantation may need to be considered.

Further studies will be required to determine whether the relatively low incidence of coronary deaths in this group of transplant patients will persist or whether a marked increase over the incidence from 1 to 3 years develops.

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