Early Postoperative Reduction of Monoclonal Antimyosin Antibody Uptake Is Associated With Absent Rejection-Related Complications After Heart Transplantation

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Background. Detection and treatment for rejection after transplantation are based on the identification of myocyte damage upon endomyocardial biopsy. Noninvasive detection of such damage is possible with \(^{111}\) In-labeled monoclonal antimyosin antibodies (MAA). Although the presence and degree of MAA uptake parallels the rejection activity detected by biopsy, the relation between the degree of uptake and the occurrence of severe rejection-related complications has not been previously assessed.

Methods and Results. Two hundred forty-seven MAA studies were performed coinciding with biopsies in 52 patients 1–71 months after transplantation. A heart-to-lung ratio (HLR) was used as a measure of relative MAA uptake, with an HLR of 1.55 discriminating normal from abnormal studies. Of the 247 antimyosin studies, 149 coincided with absent, 38 with mild, and 60 with moderate rejection at biopsy. HLR was 1.68±0.27, 1.79±0.22, and 1.91±0.33 in the three biopsy groups, respectively \((p<0.0001)\). Two hundred thirty-eight of 247 antimyosin studies coexisted with absent rejection-related complications; in nine of 247 patients, such complications were detected (five congestive heart failure episodes due to rejection and four episodes of vascular occlusion, which resulted in five deaths), and mean HLR was 1.74±0.3 and 2.1±0.16 in the two groups, respectively \((p<0.0001)\). No complications were noted in 193 studies of patients with HLR of less than 2.00, whereas in nine of 45 with HLR of 2.00 or greater, complications occurred \((p<0.0001)\).

None of the 23 patients prospectively followed since surgery who had a gradual decrease in MAA uptake during the first 3 months showed rejection-related complications, whereas persistent uptake was associated with complications in five of nine patients \((p<0.001)\).

Conclusions. No rejection-related complications are seen coinciding with HLR of less than 2.00, whereas patients who have complications have an HLR of more than 2.00. The early 3-month pattern of decreasing MAA uptake is associated with a clinical course free of rejection-related complications, whereas a persistent pattern is a signal of the possibility of such complications. (Circulation 1992;85:61–68)

Detection and treatment for rejection after transplantation are based on the identification of myocyte damage upon endomyocardial biopsies sequentially performed after surgery.\(^1\)\(^2\) The invasive nature of the procedure and the cost of its repeat application in an increasingly larger heart transplant population have led to the search for alternative methods of detecting early myocardial damage due to rejection.

Noninvasive detection of myocardial damage is


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possible with \(^{111}\text{In}\) monoclonal antimyosin antibodies.\(^3\) Binding of these antibodies to myosin takes place only when sarcolemmal disruption occurs and the cell is irreversibly damaged.\(^4,5\) Antimyosin antibodies have been successfully used in the detection of active damage in myocardial infarction,\(^6,7\) myocarditis,\(^8\) and dilated cardiomyopathy\(^9\) and during doxorubicin therapy for breast cancer.\(^10,11\)

In heart transplantation, detection of rejection-induced myocardial damage in humans was first reported by Frist et al.\(^12\) In a subsequent report, we described a semiquantitative method of assessing the degree of antimyosin uptake\(^13\) and its usefulness in recognizing variations of such uptake using repeat injections after transplantation.\(^14\) Recently, it was reported\(^15\) that following the first year after surgery, patients with negative antimyosin studies seldom show rejection at biopsy during long-term follow-up, whereas in those in whom antimyosin scans reveal uptake, there is a probability of detecting rejection that correlates with the degree of uptake. However, the relation between the intensity of antimyosin uptake and the occurrence of severe rejection-related complications has not been assessed previously; in addition, the relation between the postoperative uptake patterns and the occurrence of complications due to rejection has not been adequately studied.\(^14\) In providing the largest series to date of antimyosin studies performed in transplant patients who had long-term follow-up, the present study addresses both issues.

### Patients

From February 1987 through April 1991, 247 monoclonal antimyosin antibody studies were performed in 52 patients who were orthotopic allograft recipients. Endomyocardial biopsies were made to coincide with antimyosin studies; from one to 10 biopsy and antimyosin studies (mean, 4.7±2.1 studies) were performed 1–71 months after transplantation (mean, 15±16 months). There were 42 males and 10 females 11–62 years old (mean, 41±12 years). In 33 of these patients, repeat studies were prospectively performed at 1, 2, 3, 6, and 12 months after the surgery and yearly thereafter (prospective group). These 33 patients were the more-than-1-month survivors of a consecutive series of 53 who received an orthotopic heart transplantation; the remaining 20 died before completing the 1-month follow-up with antimyosin. The causes of death were cardiac rejection (two patients), right ventricular failure (five patients), cardiac dysfunction of unknown origin (eight patients), infection (three patients), or miscellaneous (two patients); hyperacute rejection and positive cross-match and myocardial infarction due to atherosclerosis of the donor heart.

Fourteen healthy men 22–42 years old (mean, 31±5 years) with no clinical, ECG, or echocardiographic evidence of heart disease were studied with antimyosin antibodies and constituted the control group.

### Methods

#### Immunosuppression and Detection of Rejection

Cyclosporine and steroids were administered. Antithymocytic globulin (ATGAM) was given for 10 days after transplantation. Cyclosporine doses were adjusted to whole blood levels of 400–800 ng/ml. Steroids were given in the form of methylprednisolone bolus; 1 g, 500 mg, and 500 mg methylprednisolone for the first 3 days, respectively; 1 mg/kg/day prednisone; and then reduction to 0.2 mg/kg/day. Triple therapy with azathioprine was administered to 13 patients.

Diagnosis of acute rejection was made by interpretation of endomyocardial biopsies taken with a Cordis biopate; three to five samples were taken for each procedure. Biopsy interpretation was made according to Billingham’s criteria: “normal” biopsy, “mild rejection” when cell infiltration without myocyte damage is identified, and “moderate” or “severe rejection” when different degrees of myocyte damage were detected. For the purpose of comparison with antimyosin studies, moderate and severe rejections were arranged in a single biopsy category. Treatment for acute rejection was considered only when moderate or severe rejection was detected at biopsy and consisted of a 3-day course of high-dose intravenous methylprednisolone (1 g, 500 mg, and 500 mg, respectively) and antithymocytic globulin (5 mg/kg/day with adjustment to T-cell level between 100 and 200 mm\(^3\)). Two patients required OKT3 therapy for refractory rejection. A follow-up biopsy was performed 7 days after treatment for rejection. Screening for coronary obstruction (chronic rejection) was made by coronary angiography 1 month and 1 year after transplantation, and exercise \(^{201}\text{Tl}\) myocardial perfusion studies were made every 4 months. Diagnosis of vascular rejection was made when a combination of positive \(^{201}\text{Tl}\) scan and coronary artery obstruction or positive \(^{201}\text{Tl}\) scan and small-vessel vascular obstruction at biopsy was observed. We recently reported\(^16\) that at early stages, coronary obstruction predominantly involves intramyocardial branches of coronary arteries and the more distal coronary artery bed; this corresponds to coronary vasculitis and can be reversed with a transient increase in immunosuppression. Therefore, treatment for rejection was also instituted when coronary obstruction was detected.

#### Monoclonal Antimyosin Studies

R11D10-Fab-DTPA monoclonal antimyosin Fab fragments labeled with 2 mCi of \(^{111}\text{In}\) chloride were chelated to DTPA. After giving informed consent, patients and controls were injected intradermally with 0.1 ml of the labeled antibody to test for hypersensitivity. No wheal was observed in patients or controls, and a dose of 0.5 mg R11D10-Fab-DTPA labeled with 2.0 mCi of \(^{111}\text{In}\) was administered intravenously.
Planar scintigraphic images were obtained 48 hours after antimyosin injection in anterior projection using a conventional large-field-of-view camera with a high-resolution medium-energy collimator and 20% window centered on 247- and 173-kev peaks, as previously reported from our laboratory. A minimum of 500,000 counts were collected between 5 and 10 minutes. Analog and digital images collected in a 128 x 128 matrix were stored for subsequent analysis.

**Interpretation of Antimyosin Studies**

Assessment of antimyosin uptake was made through analysis of analog images read from the unprocessed gray-scale monitor display. In the anterior projection, a four-step score was used for visual estimation: 0, no uptake; 1, mild or faint; 2, clear but moderate; and 3, intense. In addition, a heart-to-lung ratio of relative antimyosin uptake was calculated by dividing average counts per pixel in a cardiac region of interest by those obtained in a pulmonary region of interest, as previously reported. When antimyosin uptake was detected, it was usually diffuse. Occasionally, a localized area of more intense uptake with a background of a diffuse uptake was noted; in the later cases, estimation of both the visual score and calculation of the heart-to-lung ratio were made on the basis of such an area.

**Statistical Analysis**

The Kolmogorov test was used to check for normal distribution of antimyosin ratios. On the basis of the results, nonparametric tests were used. To test for differences between groups, the multiple range test using Duncan’s procedure for differences of less than 0.05 was used. Regression analysis calculated the correlation coefficient between visual score and the computed heart-to-lung ratios. \( \chi^2 \) analysis was used when appropriate.

**Results**

**Antimyosin Studies in the Control Group**

Visual estimation in the 14 controls showed absent (score of 0) or mild activity (score of 1) at the level of the left ventricle. Mean heart-to-lung ratio in the normal population was 1.43±0.06 (range, 1.30–1.50). Mean heart-to-lung ratio +2 SDs (1.55) was used as a cutoff point to discriminate normal from abnormal antimyosin studies.

**Correlation Between Visual Uptake and Antimyosin Ratios in Transplant Patients**

Correlation between visual estimation and antimyosin ratio was good \( r=0.80, p<0.0001 \). Visual scores of 0, 1, 2, and 3 correlated with increasingly higher uptake of antimyosin: 1.41±0.07, 1.61±0.15, 1.89±0.17, and 2.24±0.33, respectively. Differences were significant among all groups \( p<0.0001 \).

**Antimyosin Uptake Compared With Histological Criteria for Rejection**

Of the 247 studies, 149 coincided with absent rejection at biopsy, 38 with mild rejection, and 60 with moderate or severe rejection (Figure 1). Visual estimation scores in the three groups were 1.21±0.8, 1.55±0.72, and 1.97±0.8, and heart-to-lung ratios were 1.68±0.27, 1.79±0.22, and 1.91±0.32, respectively. Differences among each of the three biopsy score groups and between biopsy groups and controls were significant \( p<0.0001 \).

The 60 biopsies showing cellular damage (moderate or severe rejection) coexisted with abnormal antimyosin studies in 57 (95%). It is significant that in the biopsy groups with absent or mild rejection (infiltrate without apparent cell damage), a high prevalence of positive antimyosin scans was observed: 92 of 149 (62%) and 32 of 38 (84%), respectively. Taking endomyocardial biopsy as a gold standard with which results of antimyosin studies were compared, the calculated sensitivity, specificity, and accuracy of antimyosin in the diagnosis of rejection were 95%, 33%, and 31%, respectively. When antimyosin studies were taken as the gold standard with which biopsy results were compared, the calculated values were 31%, 95%, and 95%, respectively.

**Long-term Course of Antimyosin Uptake in the Global Group and the Prospectively Studied Group**

The 247 antimyosin studies were analyzed according to the time interval at which they were performed after surgery. Studies performed at 1–3 \( (n=97) \), 4–11 \( (n=39) \), 12–23 \( (n=46) \), and more than 24 months \( (n=65) \) showed a prevalence of abnormal antimyosin studies in each of the four periods of 95%, 77%, 65%, and 46%, respectively \( p<0.0001 \) (Figure 2). In these periods, mean visual estimations were 1.95±0.7, 1.33±0.7, 1.15±0.7, and 0.9±0.7, and mean heart-to-lung ratios were 1.93±0.30, 1.73±0.23, 1.65±0.22, and 1.58±0.20, respectively \( p<0.001 \).
Rejection-Related Complications

In the 33 patients who were prospectively studied since transplantation, 170 antimyosin scans were sequentially performed (mean, 4.8±1.9 scans per patient; range, 1–10 scans), and follow-up extended from 5 to 37 months (mean, 6 months). At 1–3 (n=96), 4–11 (n=38), 12–23 (n=24), and more than 24 months (n=12), prevalence of abnormal scans in each of the intervals was 95%, 76%, 58%, and 58%, respectively (p<0.0001). Mean values for visual estimation and heart-to-lung ratios in each of the intervals also decreased, to 1.95±0.7, 1.31±0.7, 1.08±0.8, and 0.9±0.9 and to 1.92±0.3, 1.72±0.23, 1.63±0.25, and 1.64±0.3, respectively (p<0.001). However, different patterns of evolution of antimyosin uptake were noted in individual patients (see Figures 3A–3E for diverse examples).

No peaks of antimyosin uptake were noted in 32 of the 33 patients prospectively studied during follow-up; therefore, no bouts of rejection ("rejection episodes") could be defined. Rather, the pattern of antimyosin uptake soon after surgery and during long-term follow-up was either a gradual reduction or persistence of such uptake. Only one patient had a clear-cut episode of enhanced rejection activity 3 months after surgery (Figure 3E).


degree of Antimyosin Uptake and Coexistence of Rejection-Related Complications

Two hundred thirty-eight of the 247 antimyosin studies in the total group coexisted without rejection-related complications; on nine occasions, such complications were detected (Table 1), with mean heart-to-lung ratios in the two groups of 1.74±0.3 and 2.1±0.16, respectively (p<0.0001). In none of 193 studies with a heart-to-lung ratio of less than 2.00 was a complication detected at the time of antimyosin imaging, whereas in nine of 45 studies with heart-to-lung ratios of 2.00 or greater, the abovementioned complications occurred (p<0.0001). Sensitivity and specificity of heart-to-lung ratio of 2.00 or greater coexisting with severe rejection-related complications were 100% and 80%, respectively; positive predictive value was 16%, and negative predictive value was 100%.

Decreasing and Persistent Patterns of Antimyosin Uptake Early After Transplantation

The patterns of evolution of antimyosin uptake during the first 3 months after transplantation and their relations to the clinical outcome are shown in Table 2. Of the 35 patients prospectively followed since transplantation, two were excluded because they died of rejection between 1 and 3 months. Of the remaining 33, a progressive reduction of antimyosin uptake with decrease of the ratio at the third month in relation to that detected at the first ("decreasing" pattern) was associated with an uneventful outcome in 21 of the 23 patients; the remaining two died as a result of suicide and carcinoma, respectively. In an additional 10 patients, such 3-month reduction in uptake after transplantation did not occur; at 3 months, the heart-to-lung ratio was equal to or higher than that obtained at the first month ("persistent" pattern). In this group, four patients died (three of acute rejection and one of vascular rejection); one patient had a reversible vascular rejection; and one patient developed heart failure from rejection that was reversed with treatment (Table 2). Differences between these two patterns in relation to development of complications were significant (p<0.001). The sensitivity and specificity of the decreasing pattern of uptake in the prediction of severe rejection-related complications (patients 3, 12, and 29 in Table 2 were excluded due to death from causes unrelated to rejection) were 100% and 87%, respectively; the positive predictive value of this pattern was 66%, and the negative predictive value was 100%.

Biopsy information from the first 3 months in the decreasing or persistent groups is shown in Table 2. The mean number of biopsies per patient during this interval in the decreasing and persistent groups were 8.5 and 8.5, respectively, and the percentages of biopsies showing myocyte damage 37% and 43%, respectively (p=NS).

During long-term follow-up, normality of antimyosin uptake was achieved in 14 patients (13 in the decreasing and one in the persistent group) a mean of 10 months (range, 2–24 months) after transplantation (Table 2), after which three patients had mild transient elevations of uptake.

Discussion

Monoclonal antimyosin antibody studies provide information regarding rejection-induced organ damage12–17 that is not available from renal, pancreas, or liver transplantation because no specific antigens have been identified in these organs against which antibodies can be synthesized. Evolution of antimyosin uptake obtained by sequential studies indicates
that no episodes of rejection, defined as bouts of activity, are actually observed in most patients but that a steady state of rejection activity occurs that usually decreases with time. This phenomenon of gradual unresponsiveness to the graft has been referred to as "tolerance." Thus, the phenomenon of tolerance appears to be a continuous, even process rather than irregular or erratic. In addition, curves of antimyosin uptake obtained in individual patients indicate that this phenomenon can be very quick in some patients and rather slow in others. In the present series, the mean interval between heart transplantation and normalization of antimyosin uptake was 10 months. However, other patients do not appear to attain complete tolerance to the graft, as antimyosin uptake is still present years after surgery. The terms "complete" or "incomplete" development of tolerance are obviously relative, as patients depend on immunosuppression.

The intensity of antimyosin uptake in relation to the occurrence of severe rejection-related complications has not been previously studied. In a recent report, the presence of antimyosin uptake after the first year of transplantation correlated with the subsequent appear-
ance of biopsy-proven rejection during follow-up. In addition, it was noted that increasing intensities of uptake correlated with a greater probability of detecting biopsy-proven rejection. Results in the present series reveal that intense antimyosin uptake, with a heart-to-lung ratio of 2.00 or greater, can be associated with complications resulting from rejection (death, coronary obstruction), whereas inferior ratios ensure against the occurrence of such complications. In addition, the early 3-month patterns of antimyosin uptake (decreasing or persistent) appear to be of useful prognostic significance. Those patients in whom antimyosin decreased during the first 3 months had an uneventful clinical course, whereas two thirds of those with a persistent pattern showed such complications. These uptake patterns provide additional information early after transplantation that is not reflected in the biopsies, as the percentages of patients showing rejection during the first 3 months were similar in the decreasing and persistent groups.

A possible explanation for the findings reported in the present study relates to a process of different degrees of active damage and clearance of antimyosin from the myocardial tissue. Recently, in a study of patients treated with doxorubicin plus antimyosin, Carrió et al.\textsuperscript{10,11} provided an adequate clinical model of acute diffuse myocardial damage to be compared with the events found in rejection. Doxorubicin produces diffuse myocardial damage that can be detected with antimyosin soon after initiation of therapy; during treatment, the higher the accumulative doses, the higher the antimyosin uptake, and patients who develop heart failure have higher antimyosin uptake than those who do not.\textsuperscript{11} These observations are in keeping with the data we report for rejection in relation to the intensity of uptake and the occurrence of severe rejection-related complications. In addition, after cessation of doxorubicin treatment, a gradual reduction of uptake toward normality is seen during the subsequent months, thus probably reflecting clearance of spilled myosin from the myocardium.\textsuperscript{11} This decreasing pattern could be equated with that found in transplant patients in whom antimyosin uptake gradually decreases to normal after several months following surgery; therefore, it can be assumed that in this group, no further important active myocardial damage as a result of rejection is present. Persistence of uptake probably reflects the presence of ongoing damage.

**Biopsy Sampling Error in Heart Transplantation**

By detecting myocyte damage, endomyocardial biopsy is used sequentially to monitor rejection and indicate treatment for rejection.\textsuperscript{1,2} However, there are data originating from this and other studies\textsuperscript{12,14,15} that suggest that biopsy sampling error could play a role in the detection of such damage. In an initial report, Frist et al.\textsuperscript{12} described two transplant patients with positive scans and negative biopsies. In a report of 54 antimyosin studies and correlations with endomyocardial biopsy results, we found 26 such discrepancies.\textsuperscript{14} In a recent report,\textsuperscript{15} results of biopsy follow-up were described in two groups of patients according to the results of antimyosin studies performed after the first year following transplantation; nine had negative antimyosin studies and negative biopsies and showed one episode of rejection during an 18-month follow-up. In 12 patients with positive scans, eight of whom presented a negative biopsy, 17 rejection episodes were detected during the same interval. This indicated that sampling error is the likely cause of these discrepancies. In the present study, when individual curves of antimyosin uptake are compared with biopsy results (see Figures 3A–3E), discrepancies appear to be due to sampling error; this corroborates the lesser sensitivity of endomyocardial biopsy compared with antimyosin studies in the detection of rejection-induced myocardial damage.

### Table 1. Antimyosin Studies in Patients With Severe Rejection-Related Complications

<table>
<thead>
<tr>
<th>Patient</th>
<th>Months after transplantation</th>
<th>Complication</th>
<th>Biopsy results</th>
<th>Antimyosin Visual HLR</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13</td>
<td>Vascular rejection</td>
<td>1</td>
<td>3</td>
<td>2.00</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>CHF</td>
<td>2</td>
<td>3</td>
<td>2.00</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>Rejection plus CHF</td>
<td>2</td>
<td>3</td>
<td>2.12</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>Rejection plus CHF</td>
<td>1</td>
<td>2</td>
<td>2.00</td>
</tr>
<tr>
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<td>3</td>
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<td>3</td>
<td>2.50</td>
</tr>
<tr>
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<td>2</td>
<td>2</td>
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<tr>
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<td>42</td>
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<td>8</td>
<td>12</td>
<td>Vascular rejection</td>
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<td>3</td>
<td>2.10</td>
</tr>
<tr>
<td>9</td>
<td>5</td>
<td>Rejection plus CHF</td>
<td>2</td>
<td>2</td>
<td>2.10</td>
</tr>
</tbody>
</table>

CHF, congestive heart failure; HLR, heart-to-lung ratio of antimyosin uptake.

*Results of endomyocardial biopsy obtained at the time of antimyosin study: 0, normal biopsy; 1, lymphocytic infiltrate without apparent cell damage; and 2, myocyte damage detected.
Antimyosin or Biopsy in Surveillance of Cardiac Rejection

These considerations raise the question of whether endomyocardial biopsy is the gold standard against which alternative, noninvasive procedures are to be tested. In addition, they pose the problem of which of the two methods to choose to detect rejection and ultimately manage transplant patients. In a previous report,\textsuperscript{14} the use of antimyosin studies during the first year after transplantation as a criterion for treatment of rejection was discouraged due to the very high sensitivity of this technique; if treatment for rejection was based on the positivity of antimyosin studies, overimmunosuppression would probably follow. In the present study, the value of antimyosin studies during the first year of transplantation is clarified; a persistent 3-month pattern of antimyosin uptake signals the possibility of rejection-related complications, whereas decreasing uptake pattern ensures against such complications. In the former situation, a closer biopsy surveillance and prompt treatment for rejection based on biopsy results would be required.

After the first year of transplantation, the role of antimyosin studies is to identify patients with normal...
studies and withdraw them from the biopsy program; biopsy surveillance is required in the remaining patients until normalcy of antimyosin is eventually achieved. It is uncertain whether long-term persistence of important antimyosin uptake (heart-to-lung ratio of 2 or greater) can be related with the development of vascular occlusion; in the present study, three patients who developed this complication had important uptake. Although identification of this subgroup of patients is of importance, further studies involving larger numbers of patients are necessary.

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References


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