

Monitoring of Cardiac Antirejection Therapy with In-111 Lymphocytes

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To determine whether lymphocytes labeled with indium-111 permit noninvasive assessment of antirejection therapy, we performed 40 allogeneic heterotopic cardiac transplants in rats. Antirejection therapy with azathioprine (30 mg/kg) and sodium salicylate (200 mg/kg) prolonged contractile function of the graft from 7.5 ± 1.5 (s.d.) days in controls to 19.4 ± 3.7 days in treated animals. Six to seven days after transplantation, autologous lymphocytes labeled with In-111 were injected intravenously in seven untreated and eight treated rats. Scintigraphy and organ counting were performed 24 hr after administration of labeled cells. At sacrifice all grafts in untreated rats exhibited contractile failure, whereas grafts in all treated rats were beating well. Transplants in untreated recipients exhibited marked accumulation of In-111 lymphocytes detectable scintigraphically, with ratios of 7.7 ± 1.9 for the activity in the transplant over that in the native heart (H_T/H_O), as obtained by well counting. In contrast, accumulation was not scintigraphically detectable in transplants of treated rats, with H_T/H_O ratios of 2.6 ± 1.8 ($p < 0.005$). The results suggest that imaging with In-111-labeled lymphocytes will permit noninvasive assessment of antirejection therapy.

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Accumulation of lymphocytes in transplanted human and animal hearts is a hallmark of rejection (1–3). Since clinical criteria such as fever and electrocardiographic changes are rather nonspecific for the early detection of rejection, it has been necessary to resort to serial endomyocardial biopsies following human cardiac transplantation (2). To determine whether early rejection and the efficacy of antirejection therapy could be assessed noninvasively, we characterized cardiac uptake of labeled lymphocytes in heterotopic heart transplants in rats. We previously reported that rejection of heterotopic cardiac allografts in rats could be detected noninvasively, based on the progressive accumulation of autologous indium-111-labeled lymphocytes (3). Thus it appeared that scintigraphic detection might be useful for noninvasive monitoring of antirejection

therapy. Accordingly, the present study was designed to determine whether the efficacy of effective antirejection therapy could be recognized by its inhibition of accumulation of labeled lymphocytes in heterotopic allografts.

METHODS

Animal preparations. Heterotopic cardiac transplantation was performed in rats using a modification of the techniques of Lee (4) and Ono and Lindsey (5). Hearts from Wistar-Furth rats* (150–250 g) were transplanted into Wistar-Lewis recipients† (175–275 g). These two strains differ at a major histocompatibility locus of the AgB system (6).

Each donor heart was removed after ligation of all veins and transection of the aorta and pulmonary artery. The coronary bed was washed with 10 ml of iced Krebs-Henseleit buffer delivered retrograde via the aorta. The aorta was then anastomosed end-to-side to the recipient's abdominal aorta, and the pulmonary ar-

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tery to the recipient's vena cava. Transplanted hearts began to beat within 1 to 3 min after unclamping of the recipient's great vessels to restore blood flow.

Labeling of lymphocytes. Lymphocytes were separated by a modification of the method of Boyum (7) and were labeled as follows (3,8). One milliliter of blood was withdrawn from the tail of the recipient rat, anticoagulated with acid-citrate-dextrose (ACD), and centrifuged at 360 g for 5 min at 22°. The leukocyte-rich plasma along with the top layer of red blood cells was aspirated, mixed with 0.5 ml of 0.9% NaCl-ACD (7:1; v/v), and layered onto 1.5 ml of Ficoll-Paque.[†] After centrifugation at 360 g for 30 min, the lymphocyte layer was removed, washed twice with NaCl-ACD, and centrifuged at 600 g twice for 10 min. Indium-111 oxine, 1.25 μ l (1 mCi/50 μ g of oxine in 50 μ l of ethanol^{||}), was diluted with 0.2 ml of NaCl-ACD and added slowly to the cell pellet. After incubation for 20 min, the suspension was centrifuged at 600 g for 10 min and the pellet washed in 0.2 ml platelet-free rat plasma (to remove free In-111 by binding to transferrin) before a repeat centrifugation at 360 g for 15 min. Three to nine million labeled cells were resuspended in 0.4 ml NaCl for injection. Efficiency of labeling was typically 35–50% (3).

Experimental protocol. Forty heterotopic cardiac transplants were performed. Twenty-one allograft transplants were studied to characterize the time course and the effects of antirejection therapy on graft survival. Subsequently, 19 additional grafts were evaluated with In-111 lymphocytes.

Effect of antirejection therapy on graft survival. Graft function was assessed at least daily by palpation and by serial electrocardiography of the transplanted heart. Completed rejection was defined as complete cessation of palpable contraction accompanied by loss of QRS amplitude and intraventricular conduction delay. Thirteen rats received no antirejection therapy (controls) and eight were given 30 mg/kg azathioprine subcutaneously daily for 12 days after transplantation and 200 mg/kg sodium salicylate subcutaneously daily throughout the entire observation interval (9).

Cardiac accumulation of In-111 lymphocytes. Accumulation of the labeled autologous lymphocytes in native and transplanted hearts was determined in seven control rats and in eight rats treated with azathioprine and sodium salicylate. On day six or seven after transplantation [established in a previous study (3) to be the time of peak rejection in untreated rats with cardiac allografts], autologous In-111-labeled lymphocytes were injected under ether anesthesia into the dorsal vein of the penis. Twenty-four hours later the animals were again anesthetized and scintigraphy performed in the anterior-posterior view with a gamma camera equipped with a medium energy collimator. Ten thousand counts were collected for each study, and immediately after data collection the animals were killed by an overdose of an-

esthesia. Activity in the native and transplanted hearts and in blood was quantified in a gamma well counter. Three to four transverse sections of each transplant were fixed in calcium acetate buffered 10% formalin, embedded in paraffin, sectioned at 5 μ m, stained with hematoxylin and eosin, and examined by optical microscopy.

Four additional rats with allografts were treated with azathioprine/salicylate and injected with labeled lymphocytes at day 3 (n = 2) or day 5 (n = 2) to determine the time course of accumulation of activity in treated grafts. Twenty-four hours after administration of labeled cells, the rats were killed for analysis of organ radioactivity.

Statistics. Values expressed are mean \pm standard deviation. Student's t-test for nonpaired observations was used for analysis of differences between treated and untreated animals.

RESULTS

Effects of antirejection therapy on survival of transplanted hearts. Responses to antirejection therapy with azathioprine and sodium salicylate are shown in Fig. 1.

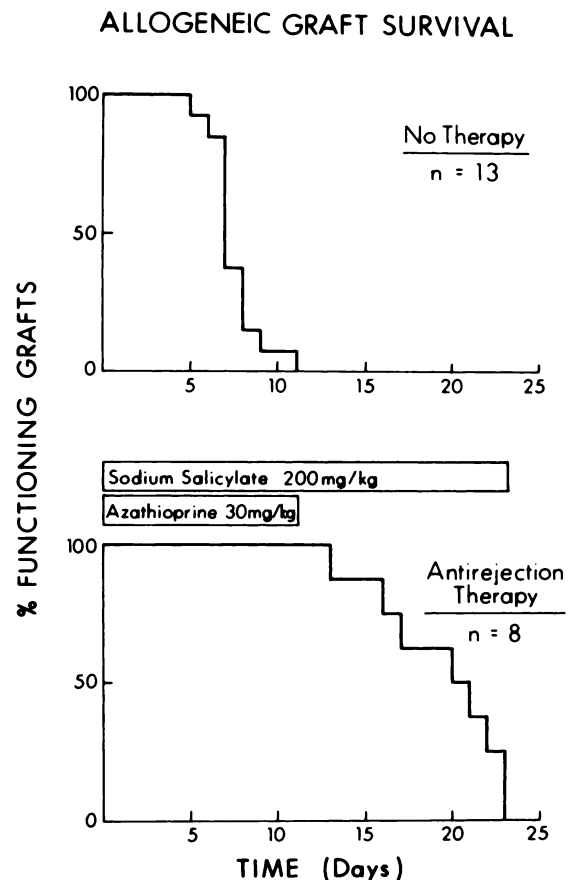


FIG. 1. Cumulative representation of contracting grafts as a function of time after transplantation in treated and untreated recipients. Graft contractile function was assessed by daily palpation. Loss of graft function was defined as complete cessation of palpable contraction.

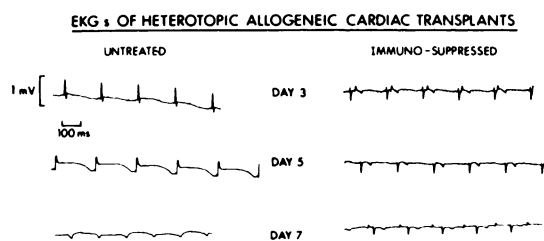


FIG. 2. Representative serial electrocardiograms of allografts from treated and untreated animals. Untreated rats show progressive decreases in heart rate, with conduction delay and diminution of QRS amplitude. In contrast, immunosuppressed animals show no marked changes in these parameters over corresponding intervals.

In untreated rats, cessation of contraction of the allogeneic, heterotopic transplant occurred between the fifth and eleventh day after surgery, with a mean graft survival of 7.5 ± 1.5 days. In rats given antirejection therapy, all grafts were beating more than 12 days after transplantation and mean graft survival was 19.4 ± 3.7 days ($p < 0.001$).

Serial electrocardiograms of allografts in untreated animals showed conduction delays, progressive diminution of heart rate, and eventual intraventricular block (Fig. 2). In contrast, allografts in treated rats showed no change in rate and no conduction abnormalities over corresponding intervals (Fig. 2).

Accordingly, In-111 lymphocyte uptake studies described below were performed during an interval in which a high incidence of functional cardiac failure due to rejection is typical in untreated animals but not in treated animals.

Effects of antirejection therapy on cardiac accumulation of In-111 lymphocytes. Table 1 indicates the time course of accumulation of radioactivity in allografts from treated and untreated animals, compared with accu-

SCINTIGRAMS OF 8 DAY HEART TRANSPLANTS IN RATS

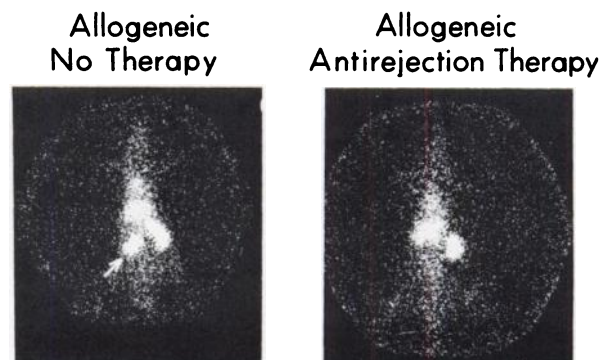


FIG. 3. Anterior scintigrams obtained 24 hr after intravenous injection of autologous In-111-labeled lymphocytes in treated and untreated rats. Marked accumulation of activity in transplant is visible in scintigram from untreated rat. Arrow indicates allograft. In contrast, no accumulation is visible in grafts of rats receiving antirejection therapy.

mulation in each animal's own heart. As reported previously, allografts accumulate some radioactivity 3-4 days after transplantation, probably owing to inflammation arising from the surgical procedure (3,10). In allografts from treated rats, accumulation increased minimally thereafter. In contrast, allografts from untreated rats exhibited progressive and marked accumulation of abel (Table 1).

Figure 3 shows scintigrams obtained eight days after transplantation and 24 hr after intravenous administration of In-111-labeled autologous lymphocytes in rats with and without antirejection therapy. In the image the cardiac transplant in the control animal (no antirejection

TABLE 1. COMPARISON OF ACCUMULATION OF ¹¹¹In-LABELED LYMPHOCYTES BY CARDIAC ALLOGRAFTS IN UNTREATED AND IMMUNOSUPPRESSED RATS AT SELECTED INTERVALS AFTER TRANSPLANTATION. H_T = TRANSPLANTED HEART; H_O = NATIVE HEART, BLD = BLOOD. ALL RATIOS ARE NORMALIZED PER GRAM TISSUE. UPTAKE REFERS TO THE UPTAKE OF RADIOACTIVITY OF THE ENTIRE H_T AS A PERCENTAGE OF INJECTED RADIOACTIVITY. VALUES INDICATE THE MEAN AND (RANGE).

Days after transplantation (n)	H _T /H _O	H _T /BLD	Uptake (%)
Untreated group			
3-4* (3)	1.4 (0.8-2.4)	3.0 (0.1-0.5)	0.4 (0.3-0.5)
5-6* (11)	6.5 (3.5-8.0)	1.2 (0.5-3.5)	3.1 (0.7-5.3)
7-8 (7)	7.7 (4.8-10.)	3.4 (0.8-6.1)	1.4 (0.6-2.7)
Immunosuppressed group			
3-4 (2)	1.7 (1.1-2.2)	0.3 (0.2-0.4)	0.5 (0.3-0.7)
5-6 (2)	1.9 (1.5-2.4)	0.4 (0.3-0.5)	0.5 (0.3-0.7)
7-8 (8)	2.6 (0.6-5.1)	0.5 (0.1-1.1)	0.2 (0.1-0.3)

* Data at this interval for untreated rats have been reported previously (3), but are included for purposes of comparison.

therapy) is visible as a hot spot under the liver in the right lower abdominal quadrant. In contrast, no appreciable accumulation of activity is visible in the region of the abdominal, heterotopic allograft in the rat treated with antirejection therapy.

Figure 4 shows cardiac In-111 activity determined by well counting at Day 8 after transplantation. The ratio of activity per gram myocardium in the transplant compared with that in the native heart (H_T/H_O) in untreated animals averaged 7.7 ± 1.9 , the percentage of the injected dose per gram of allograft myocardium (%ID) averaged $1.4 \pm 0.6\%$, and the tissue-to-blood activity ratio averaged 3.4 ± 2.1 . In contrast, transplants in treated recipients exhibited an H_T/H_O ratio of 2.6 ± 1.8 , %ID of 0.2 ± 0.1 , and a tissue-to-blood ratio of 0.5 ± 0.3 ($p < 0.005$ for each of the three comparisons between groups). Microscopic sections from transplants from untreated rats exhibited intense infiltration with lymphoid cells, severe destruction of cardiac muscle fibers, and interstitial hemorrhage virtually throughout the left and right ventricular walls. Changes were qualitatively similar but much less intense and less widespread in transplants from treated rats, occurring only in a patchy distribution and with extensive regions of histologically normal muscle interspersed between apparent lesions.

DISCUSSION

Indium-111-labeled lymphocytes are particularly suitable for noninvasive assessment of lymphocyte accumulation in rejecting organs because they remain viable in vivo for several days, the In-111 energy spectrum is suitable for gamma imaging, and the In-111 half-life (2.8 days) is long enough for imaging even after several days but short enough to prevent an excessive

radiation burden (11,12). In a previous study of heterotopic heart transplants in rats, we demonstrated that In-111-labeled lymphocytes accumulate progressively in myocardium of rejecting allografts, although activity in isogeneic control transplants did not differ from activity in the animal's native heart (3). The results of the present study demonstrate that antirejection therapy sufficient to prolong cardiac graft survival results in a significant diminution of accumulation of In-111-labeled lymphocytes compared with accumulation in grafts in untreated animals, detectable not only by analysis of the tissue in vitro but also by antemortem scintigraphy.

The antirejection therapy protocol was based on its previously proven efficacy in allogeneic cardiac transplants in rats (9). In the present study grafts of treated animals were beating well at the time of scintigraphy. Nevertheless, patchy lymphocyte infiltration indicative of an incipient rejection process was detected histologically in allografts from treated rats. Accordingly, it is not surprising that In-111 activity quantitated by well counting in grafts from treated animals exceeded activity in the native hearts, in contrast to results in isogeneic transplants (3).

One potentially complicating factor in studies with In-111-labeled lymphocytes is contamination of conventional lymphocyte preparations with platelets (13). It could be argued that the decreased In-111 activity in grafts of treated animals reflects, in part, diminished aggregation and accumulation of In-111-labeled platelets, rather than decreased accumulation only of lymphocytes. The dose of sodium salicylate administered in the present study, however, does not inhibit platelet aggregation in rats (14). In addition, the ratio of activity in a rejecting graft compared with that in the native heart is ≥ 7.0 even when a highly purified platelet-free lymphocyte preparation is used (3). Accordingly most, if not all, of the decrease of radioactivity in grafts from treated animals in the present study appears due to decreased accumulation of lymphocytes.

Results of this study suggest that cardiac imaging after intravenous injection of In-111-labeled lymphocytes is a potentially useful approach not only for early noninvasive detection of rejection in patients undergoing cardiac transplantation, but also for noninvasive monitoring of the efficacy of antirejection therapy.

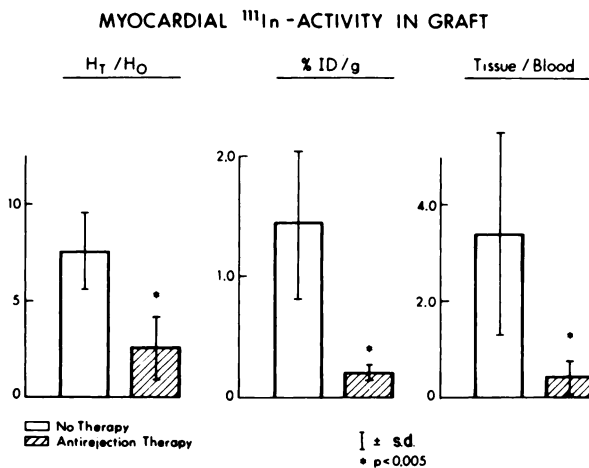


FIG. 4. Effect of antirejection therapy on myocardial uptake of In-111-labeled lymphocytes, as determined by gamma well counting. H_T/H_O = ratio of activity per gram myocardium in transplanted compared with native heart. % ID/g = activity per gram myocardium in percent of injected dose. (n = 7 in untreated group, 8 in immunosuppressed group.)

FOOTNOTES

- * Microbiological Associates.
- † Charles River.
- ‡ Pharmacia.
- § Diagnostic Isotopes, Inc.

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ABSTRACT BOOKS

Owing to the popularity of the 1981 *Abstract Book* the 1982 *Abstract Book*, including all abstracts accepted for the 29th Annual Meeting of the Society, will be available for \$5.00 in advance and at the Meeting June 15-18, 1982 in Miami Beach, FL. Advanced copies may be ordered on the Meeting Registration form.

ERRATUM

In the article entitled "Evaluation of Inorganic Absorbents for the Rubidium-82 Generator: I. Hydrous SnO₂" (*J Nucl Med* 23:245-249, 1982), some of the authors were omitted inadvertently. The complete author listing should read: R.D. Neirinckx, J.F. Kronauge, G.P. Gennaro, M.D. Loberg, and the Los Alamos Medical Radioisotope Group.