Noninvasive detection of rejection of transplanted hearts with indium-111–labeled lymphocytes*

HOWARD J. EISEN, M.D., STEVEN B. EISENBERG, M.D., JEFFREY E. SAFFITZ, M.D., PH.D.,
R. MORTON BOLMAN III, M.D., BURTON E. SOBEL, M.D., AND
STEVEN R. BERGMANN, M.D., PH.D.

With the technical assistance of James E. Bakke, M.S.

ABSTRACT  To determine whether cardiac transplant rejection can be detected noninvasively with indium-111 (111In)–labeled lymphocytes, we studied 11 dogs with thoracic heterotopic cardiac transplants without immunosuppression and five dogs with transplants treated with cyclosporine (10 mg/kg/day) and prednisone (1 mg/kg/day). All were evaluated sequentially with gamma scintigraphy after administration of 150 to 350 μCi of autologous 111In-lymphocytes. Technetium-99m–labeled red blood cells (1 to 3 mCi) were used for correction of radioactivity in the blood pool attributable to circulating labeled lymphocytes. Lymphocyte infiltration was quantified as the ratio of indium in the myocardium of the transplant or native heart compared with that in blood (indium excess, IE). Results were correlated with mechanical and electrical activity of allografts and with histologic findings in sequential biopsy specimens. In untreated dogs (n = 11), IE was 15.5 ± 7.0 (SD) in transplanted hearts undergoing rejection and 0.4 ± 1.1 in native hearts on the day before animals were killed (p < .01). In dogs treated with cyclosporine and prednisone (n = 5), IE was minimal in allografts during the course of immunosuppression (0.8 ± 0.4) and increased to 22.9 ± 11.1 after immunosuppression was stopped. Scintigraphic criteria of rejection (IE > 2 SD above that in native hearts) correlated with results of biopsies indicative of rejection and appeared before electrophysiologic or mechanical manifestations of dysfunction. Thus infiltration of labeled lymphocytes in allografts, indicative of rejection, is detectable noninvasively by gamma scintigraphy and provides a sensitive approach potentially applicable to clinical monitoring for early detection of rejection and guidance for titration of immunosuppressive measures.


CARDIAC TRANSPLANTATION is an increasingly important treatment for end-stage cardiac disease, but rejection continues to be a major complication. Criteria for the presence of rejection — fever, atrial arrhythmia, alterations in QRS voltage, leukocytosis, and impaired ventricular performance — are insensitive and nonspecific. Thus serial endomyocardial biopsy is required for monitoring patients. Although relatively safe, biopsy is an invasive and expensive approach to detecting rejection.

Infiltration of lymphocytes is a feature of early rejection.2–4 We and others have demonstrated the feasibility of detecting early rejection of heterotopic transplants in small laboratory animals with indium-111 (111In)–labeled lymphocytes.5–8 Detection of rejection with 111In-leukocytes has also been attempted in patients.9 However, the suitability of this approach for potential clinical applications has not been established. The present study was performed to determine whether rejection of heterotopic cardiac transplants in large animals under conditions simulating those encountered clinically could be detected sensitively and reliably. Erythrocytes labeled with technetium-99m (99mTc) were used as a blood pool tracer for compensation by subtraction of radioactivity attributable to circulating lymphocytes. The subtraction technique is analogous

From the Cardiovascular Division, Department of Internal Medicine, and Division of Cardiothoracic Surgery, Department of Surgery, Washington University School of Medicine, St. Louis.

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Address for correspondence: Steven R. Bergmann, M.D., Ph.D., Cardiovascular Division, Washington University School of Medicine, 660 S. Euclid Ave., Box 8086, St. Louis, MO 63110.

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to one developed in our laboratory for the scintigraphic identification of coronary artery thrombi. Each dog was used as its own control with correlative assessments of scintigrams and histopathologic studies in the native and transplanted hearts. Some dogs were treated with immunosuppressive agents to define the utility of scintigraphy for detecting effects of treatment.

**Methods**

**Animals.** Sixteen mongrel dogs were subjected to chest heterotopic cardiac transplantation. They were anesthetized with sodium pentothal and intubated. Ventilation was maintained with a Harvard respirator. Anesthesia was maintained with enflurane (Ohio Medical Products). Donor hearts harvested from unmatched mongrel dogs were arrested with hyperkalemic cardioplegic solution and placed in the recipient’s chest via a left thoracotomy. The donor aorta was anastomosed to the recipient subclavian artery and the donor pulmonic artery to the recipient superior vena cava in an end-to-side fashion. Thus circulation to the allograft was retrograde, perfusing only the grafts via their coronary circulation. The allografts did not support the recipient’s circulation.

**Experimental protocol.** Eleven dogs were given prednisone perioperatively without other immunosuppressive agents. Spontaneous rejection of the allografts occurred in each. Electrocardiograms with six limb leads and fluoroscopic assessments of allograft function were obtained daily. Lymphocytes were labeled 3 days after transplantation. Scintigraphy was performed daily beginning 24 hr after administration of labeled lymphocytes and continued until mechanical activity of the allograft ceased. Lymphocytes were labeled approximately every 3 to 4 days so that acquisition of counts would be sufficient (1/2 of 67 hr). Right ventricular endomyocardial biopsies were performed transvenously under fluoroscopic guidance every 3 days with a cardiac biopomé (Cordis).

Five other animals with transplants were subjected to an immunosuppressive regimen for 2 weeks consisting of oral administration of cyclosporine (10 mg/kg/day) and prednisone (1 mg/kg/day). After 2 weeks, immunosuppression was stopped so that allograft rejection would occur. Endomyocardial biopsy specimens and scintigrams were obtained every 3 days. All animals, i.e., those with and without immunosuppression, were killed as soon as possible after cessation of mechanical function of the allografts.

**Scintigraphy.** Dogs were anesthetized with thiopental and placed in the right lateral decubitus position. Scintigrams were obtained with a Siemens gamma camera with a medium-energy 4000 parallel-hole large-field-of-view collimator set for both gamma energy peaks of 111In (171 and 247 keV) with a 20% window. Between 150,000 and 450,000 counts were obtained generally within 15 to 45 min depending on administered dose and decay of indium radioactivity. Five minutes later, the gamma camera was set to detect the major peak of 99mTc (140 keV) with a 20% window. A total of 225,000 to 450,000 counts was obtained, generally within 3 to 5 min. No spillover correction (from indium to technetium) was used because the amount of 99mTc activity injected exceeded the 111In activity injected by fivefold to 20-fold and because the amount of cross-over from 111In into the 99mTc window was modest (9%). Contributions from radioactivity in the liver and spleen were minimized by positioning of the camera. No shielding was used. Total time for scanning was generally 30 to 40 min. Data were stored on floppy disks for subsequent analysis.

**Analysis of scintigrams.** Index excess (IE), the excess radioactivity attributable to accumulation of lymphocytes in a selected region of interest relative to radioactivity in blood, was estimated as previously described:

$$IE = I_{TOT} - I_{BP}$$

where $I_{BP} = (I_{REF}/T_{REF})$ and $I_{TOT} = total^{111}In radioactivity in a region of interest of the native or transplanted heart, $I_{BP} = 111In$ in blood in the region of interest, $I_{REF}/T_{REF} = relative activity of the two radiotracers in blood in a reference region, and $T_{REF} = 99mTc$ activity in region of interest.

The regions of interest over native and transplanted hearts were selected from the 99mTc scintigrams that delineated the blood pool well. Regions were drawn over the entire cardiac contour and generally comprised 40 to 90 pixels (of a 64 x 64 pixel matrix). A reference region within which blood pool activity was high but deposition of lymphocytes unlikely was chosen generally over the ascending or descending aorta and occasionally over the carotid or subclavian arteries. We have shown previously that $I_{REF}/T_{REF}$ is virtually identical in these three regions. Analysis of data was performed with an ADAC II computer by an observer blinded to the results of biopsy.

**Labeling of lymphocytes.** Sixty milliliters of whole blood was obtained from each recipient. A large volume (90 ml) was obtained from animals treated with cyclosporine to compensate for the decline in lymphocyte count with treatment. Blood was anticoagulated with acid-citrate-dextrose (ACD) (10:1, vol/vol). Samples contained 6000 to 18,000 white blood cells/mm³ with 15% to 25% lymphocytes in untreated dogs and 10% to 18% lymphocytes in treated dogs. Fifteen milliliter aliquots were layered on 15 ml Ficolli-Paque (Pharmacia) in 50 ml polystyrene centrifuge tubes (Corning) and centrifuged (IEC Centra 7-R) at 360 g at 25°C for 30 min. The lymphocyte layer was removed and washed twice in 50 ml polycarbonate centrifuge tubes (Sorvall) with 0.9% NaCl-ACD (7:1, vol/vol) by centrifugation at 600 g at 25°C for 10 min (Sorvall RC-5 centrifuge). The resulting pellet was diluted with 1 ml of platelet-free plasma and 2 ml of NaCl-ACD, layered onto 8 ml Ficolli-Paque in 15 ml polystyrene centrifuge tubes (Corning), and centrifuged at 360 g for 30 min. The lymphocyte layer was then washed twice in NaCl-ACD at 600 g for 10 min followed by a wash in 1 ml platelet-free plasma and one in NaCl-ACD at 600 g for 10 min. Recovery of lymphocytes in the final cell pellet averaged 57 ± 12% (mean ± SD). The second Ficolli-Paque centrifugation was used to reduce contamination to less than four platelets per lymphocyte in the final preparation.

The final cell pellet, containing 2 to 5 x 10⁹ cells, was suspended in 1.0 ml of NaCl-ACD. One hundred fifty to 300 μCi In-oxine (Amersham) (containing a maximum of 2.4 mg of HEPES buffer and 20 μg of oxine per dose) was added slowly to the suspension, incubated at room temperature for 20 min, and centrifuged at 600 g for 10 min. To remove unbound In-oxine, labeled cells were washed twice in 1 ml of platelet-free plasma at 600 g for 10 min in a Dynac II centrifuge. Efficiency of labeling, determined by counting radioactivity in 2 μl aliquots of the initial and final suspension and in all resulting supernatants in a gamma-well counter (LKB-Wallace, Model 1282), was 45% to 85%. The cells were suspended in 2 ml of NaCl-ACD and injected intravenously. Viability, as assessed by the nigrinos exclusion test, was 97%.

To determine whether the indium label remained associated with circulating lymphocytes in vivo, 24 hr after administration of labeled lymphocytes, 2 ml of whole blood were obtained and centrifuged at 600 g for 5 min to separate platelet-rich plasma (PRP) from lymphocytes and other cellular elements. The PRP was centrifuged at 3000 g for 15 min to separate platelets from plasma. Aliquots of whole blood, PRP, and platelet-poor plasma were assayed for radioactivity in a gamma-counter. A total of 80% to 95% of radioactivity was recovered in the cellular fraction, 2% to 5% in plasma (as indium-transferrin), and
5% to 12% in platelets (correcting for platelets/mm³ in PRP compared with whole blood).

Labeling of erythrocytes with ⁹⁹ᵐTc. To permit subtraction of radioactivity attributable to labeled lymphocytes circulating in the blood pool, we employed a subtraction technique with ⁹⁹ᵐTc-labeled red blood cells. For labeling, 2 ml of whole blood were obtained and anticoagulated with ACD. The blood was centrifuged at 1800 g for 2 min. The supernatant fraction was removed, and the cells were washed once with 0.9% NaCl at 1800 g at 22°C for 2 min. The erythrocytes were incubated with 50 µg of stannous glucoheptonate for 5 min followed by an NaCl wash and then incubated with 3 to 4 mCi of ⁹⁹ᵐTc pertechnitiate at 22°C for 5 min. The cells were washed and resuspended in 2 ml NaCl. Efficiency of labeling was 80% to 95%. ⁹⁹ᵐTc-labeled erythrocytes were administered intravenously after acquisition of data with ¹¹¹In.

Histology and postmortem well counting. Biopsy and postmortem samples were fixed in sodium phosphate–buffered 10% formalin. Samples were stained with hematoxylin and eosin for histologic assessment by an investigator blinded to scintigraphic data and treatment regimen. Histologic grading was performed according to theBillingham classification.¹¹ Radioactivity in postmortem specimens of native and transplant hearts and blood was assayed in a gamma-well counter after the specimen had been weighed and fixed in formalin.

Autoradiography. To confirm that infiltration of radioactivity into allografts was associated with labeled lymphocytes, autoradiographs were prepared from frozen sections of myocardium from an untreated allograft recipient. Sections were applied to gelatin-coated slides, dipped into Kodak nuclear track emulsion (type NTB-3, 1:1 dilution in water) at 32°C, and withdrawn at a constant rate of 120 mm/min. The slides were then dried in the vertical position, placed in light-tight plastic boxes containing silica gel desiccant, and stored at 4°C. Twenty-one to 28 days later, the slides were warmed to 20°C and developed in Kodak D-19 developer, distilled water, and fixer. Slides were rinsed three times in deionized water and stained with hematoxylin and eosin.

Statistical analysis. Values were expressed as means ± SD. Paired t tests were used for comparisons between transplant and native hearts. A t test for independent samples was used for comparisons between treated and untreated allografts; p < .05 defined significant differences.

Results

Sixteen heterotopic transplants were successful. Postmortem analysis was performed as soon as possible after cessation of mechanical activity of the rejecting allograft was detectable fluoroscopically. The 11 recipients not treated with immunosuppression were killed a mean of 10 ± 4 days after surgery (range 6 to 18) (figure 1A). Five dogs were treated with cyclosporine (10 mg/kg/day) and prednisone (1 mg/kg/day) for 2 weeks after transplantation. Mean survival of the allografts in this group was 26 ± 12 days (figure 1B).

All initially successful heterotopic transplants exhibited normal sinus rhythm 1 day after operation. Rejecting hearts manifested progressive bradycardia, decreased QRS voltage, and widening of the QRS complex. No electrophysiologic evidence of rejection was noted in hearts from dogs being treated with immunosuppressive agents.

Accumulation of radioactivity in untreated allografts. Scintigrams obtained after administration of ¹¹¹In-lymphocytes and ⁹⁹ᵐTc-labeled red blood cells and after correction for circulating lymphocyte activity from a dog in the untreated group are shown in figure 2. Four days after transplantation, no uptake of ¹¹¹In-lymphocytes was noted in either the allograft or the native heart. Two days later, uptake was observed in the rejecting allograft. With correction for radioactivity associated with lymphocytes circulating in the blood pool, uptake of ¹¹¹In-lymphocytes in the allograft was accentuated.

¹¹¹In accumulation in allografts increased with time after transplantation in all untreated dogs as shown in figure 3. No significant accumulation was evident in the native hearts (figure 3 and table 1).

Effect of immunosuppression on accumulation of ¹¹¹In-lymphocytes in transplanted hearts. ¹¹¹In scintigrams, ⁹⁹ᵐTc scintigrams, and blood pool–corrected scintigrams obtained 24 hr after injection of ¹¹¹In-lymphocytes from a dog treated with cyclosporine and prednisone for 12 days and in the same animal 5 days later (2 days after cessation of immunosuppression) are shown in figure 4. Marked accumulation of ¹¹¹In activity in the
allograft occurred when immunosuppression was stopped.

Accumulation of $^{111}$In activity during and after cessation of immunosuppression in all dogs in the treated group is summarized in figure 5 and table 2. Accumulation of lymphocytes in the allografts did not occur during immunosuppression but was progressive after its cessation. No significant uptake of $^{111}$In occurred in native hearts.

Identification of rejection by scintigraphy. Scintigraphic evidence of rejection (defined as an IE > 2 SD above that in native hearts, i.e., > 2.6) was compared with histologic degree of rejection. Seventeen episodes of rejection were documented by endomyocardial biopsy. Of four mild episodes of rejection, three were correctly identified scintigraphically. Seven episodes of moderate rejection and six episodes of severe rejection were documented by endomyocardial biopsy and all of these were correctly identified scintigraphically. For all 18 histologically normal biopsy specimens, concurrent scintigrams were read as normal. Three specimens were read as equivocal. One of the hearts from which they came exhibited scintigraphic evidence of rejection.
The extent of uptake of $^{111}$In assayed postmortem by well counting is shown in table 3. High ratios of radioactivity in transplant compared with native hearts with respect to radioactivity in blood were evident.

**Autoradiography.** Autoradiographs from a cardiac allograft obtained 7 days after transplantation from an untreated dog demonstrated that accumulation of radioactivity was attributable to labeled lymphocytes (figure 6).

**Discussion**

Results of this study indicate that $^{111}$In-labeled lymphocytes accumulate in rejecting cardiac allografts progressively and that the uptake is readily detectable by sequential gamma scintigraphy. Subtraction of radioactivity associated with labeled, circulating lymphocytes with the use of $^{99m}$Tc-labeled red blood cells permitted assessment of infiltration of allografts with labeled lymphocytes. Immunosuppression prevented both rejection and infiltration of labeled lymphocytes reflected scintigraphically. Cessation of immunosuppression elicited rejection detectable scintigraphically by increases in uptake of $^{111}$In-lymphocytes in allografts and confirmed histologically.

The mean IE in native control hearts was $0.4 \pm 1.1$ (range 0 to 3.7). An IE over 3.7 did not occur in any native heart or in allografts during immunosuppression. Allografts with an IE greater than 4.0 exhibited rejection histologically. Only one exception occurred with a biopsy interpreted as “equivocal” from a heart with an IE of 5.7 when immunosuppression was stopped. Because early rejection is often patchy, this allograft may have been undergoing rejection.

Lymphocytes are the first cellular elements to infiltrate myocardium undergoing rejection.\(^3\text{-}^4\) Despite possible release of label from lymphocytes in vivo, 90% of radioactivity in blood 24 hr after administration was still associated with lymphocytes. Autoradiography confirmed that radioactivity noted in rejecting allografts was associated with lymphocytes.

Labeled lymphocytes migrate into rejecting cardiac transplants in rats\(^5\text{-}^8\) and dogs.\(^1^4\) However, rejection may not be detected if radiation impairs lymphocyte function or if the delay between injection of labeled lymphocytes and the onset of rejection is substantial. $^{111}$In-labeled leukocytes have been used for detection of graft rejection in patients,\(^9\) but in that study the volume of blood used for harvesting cells was limited (7 ml) and lymphocytes were not separated from other cellular elements.

Because infiltration with lymphocytes is the earliest histologic manifestation of rejection, sensitive detection of this process may provide the best opportunity for diagnosing rejection at its outset. The use of labeled lymphocytes as markers for detection of rejection before necrosis should facilitate titration of antirejection therapy that can still be protective.

Lymphocytic infiltration of myocardium unrelated to allograft rejection may be seen in certain clinical situations that may occur in the transplant population. Biopsies performed shortly after transplantation may reveal evidence of ischemia-induced reperfusion injury. Histologically this is manifested by subendocardial leukocytic infiltration. Scintigraphically, it may be difficult to differentiate from rejection in the early

**TABLE 1**

<table>
<thead>
<tr>
<th>Time of procedure after transplantation</th>
<th>Native heart</th>
<th>Transplanted heart</th>
</tr>
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<tbody>
<tr>
<td>Initial study after transplantation</td>
<td>0</td>
<td>$0.1 \pm 0.3$ (0-0.8)</td>
</tr>
<tr>
<td>Day before complete rejection</td>
<td>4</td>
<td>$0.2 \pm 0.5$ (0-0.9)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>$3.2 \pm 2.8$ (0.8-8.4)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>$9.8 \pm 6.0$ (3.9-23.9)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>$15.5 \pm 7.0$ (7.7-30.8)</td>
</tr>
</tbody>
</table>

Data expressed as mean $\pm$ SD (and ranges) of IE in native hearts and transplanted hearts at the time of the initial study and from 1 to 4 days before death. ($n = 11$).\(^a\)p < .01.
FIGURE 4. Scintigrams obtained from a dog subjected to immunosuppression. A to C, Scintigrams of total indium activity (A), $^{99m}$Tc red blood cells (B), and the subtraction scintigram obtained 12 days after transplantation and during the course of immunosuppression (C). No IE is noted in the allograft ($N =$ native heart, $A =$ allograft). D to F, Corresponding scintigrams obtained 5 days later, and 3 days after immunosuppression had been stopped. IE is evident in the allograft in the indium scintigram alone (D) and accentuated in the subtraction image (F). The IE in the allograft was 23.7.

postoperative period, although several factors may help in distinguishing the two processes. These include the nature of the cellular infiltrate, the time course of reperfusion injury compared with cell-mediated rejection (the former occurs within the first 48 hr after surgery while the rejection occurs later), and the histologic distribution of the cellular infiltrate (subendocardial in ischemia vs diffuse in rejection). Transplant patients may also develop opportunistic infections that may involve the myocardium, most often due to toxoplasmosis or cytomegalovirus, in which lymphocytic infiltration is frequently evident histologically. Scintigraphy with labeled lymphocytes may not be able to distinguish these syndromes from acute rejection. Endomyocardial biopsy would be required to assist in diagnosing these infections, although the characteristic histologic features of toxoplasmosis (cysts) or cytomegalovirus (giant cells with inclusion bodies) may be absent. Supplemental clinical data such as serologic tests, virus isolation from cultures of buffy coat and urine, and evidence of systemic disease are often required to confirm these diagnoses. Mononuclear cell infiltration can be present in histologic specimens of nonrejecting allografts in cyclosporine-treated patients. This has been raised as a potential drawback for using labeled lymphocytes to detect rejection. Widely dispersed interstitial mononuclear infiltrates may be seen soon after transplantation in cyclosporine-treated patients who are not rejecting their transplants but not in those treated with...
azathioprine, antilymphocyte globulin, and steroids. Nevertheless, histologic changes of early, acute rejection are similar in cyclosporine-treated patients and in those treated with more traditional regimens.\textsuperscript{13} The hallmark of rejection is perivascular and endocardial infiltrates of mononuclear cells, quantitatively and qualitatively different from the changes seen in nonrejecting allografts from cyclosporine-treated recipients.

Despite its high cost and invasive nature, endomyocardial biopsy is the present procedure of choice for detecting rejection in patients with heart transplants. As the number of patients receiving cardiac transplants increases, noninvasive methods for detecting rejection are becoming increasingly important.

Other approaches with thallium-201 or $^{99m}$Tc-pyrophosphate for detection of graft rejection in animals\textsuperscript{19-22} and in patients\textsuperscript{23} have been reported, but their sensitivity and specificity have yet to be established. Scintigraphy after administration of $^{111}$In-labeled antimyosin monoclonal antibodies have been promulgated by several groups for detection of allograft rejection.\textsuperscript{24, 25} Although labeled antimyosin antibodies may detect grade III rejection, where necrosis of myocytes is extensive, their utility for detection of grade I or even grade II rejection (in which only focal myocytic damage is present) may be limited. Furthermore, antimyosin antibody is accumulated only by cells that are already irreversibly damaged.\textsuperscript{26}

With some approaches, for example those using antimyosin antibodies, the dose of $^{111}$In used delivers a significant radiation burden to kidneys, liver, and other organs.\textsuperscript{11} In scintigraphy with blood pool subtraction as implemented in the present study required injection of 100 to 200 $\mu$Ci (as opposed to 1 to 3 mCi with $^{111}$In-antimyosin antibodies) of $^{111}$In. Although 3 to 4 mCi of $^{99m}$Tc-labeled red blood cells were used as well, the half-life of this tracer is only 6 hr. Thus the radiation burden was modest ($\sim 0.08$ rad/total body). Nonetheless, the clinical utility of $^{111}$In-labeled lymphocytes, particularly for sequential studies, may be limited by the radioactive burden on organs, including the liver, spleen, and kidney.\textsuperscript{11} In may damage chromosomes in lymphocytes and impair lymphocyte function,\textsuperscript{18, 27, 28} but no evidence for malignant transformation has been noted.\textsuperscript{27} In addition, oxine and alcohol, congeners of commercial indium oxine, can damage lymphocytes if used in excessive concentrations.\textsuperscript{29}

Thakur and McAfee\textsuperscript{27} estimated the doses of radioac-

### TABLE 2

<table>
<thead>
<tr>
<th>Time of procedure</th>
<th>Scintigraphic IE</th>
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<tr>
<td></td>
<td>Native heart</td>
</tr>
<tr>
<td><strong>Day after transplantation</strong></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>0.1 ± 0.2 (0–0.3)</td>
</tr>
<tr>
<td>11</td>
<td>0.4 ± 0.5 (0–0.7)</td>
</tr>
<tr>
<td>14</td>
<td>0.1 ± 0.2 (0–0.3)</td>
</tr>
<tr>
<td><strong>Day before complete rejection</strong></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.9\textsuperscript{a}</td>
</tr>
<tr>
<td>4</td>
<td>0.6 ± 0.6 (0 ± 1.2)</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Data expressed as mean ± SD (and ranges) of IE in transplanted hearts and native hearts in dogs treated with cyclosporine and prednisone for 14 days. After cessation of immunosuppression, a progressive increase of IE in the allografts occurred, reaching a maximum 1 day before death (n = 4).

\textsuperscript{a}P < .01.

\textsuperscript{a}Only one animal in the group survived more than 7 days after immunosuppression was stopped.
Radioactivity in samples assayed postmortem by well counting

<table>
<thead>
<tr>
<th>Tissue ratios</th>
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<tbody>
<tr>
<td>Transplant LV/native LV</td>
<td>13.7 ± 8.1</td>
</tr>
<tr>
<td>Transplant RV/native RV</td>
<td>14.4 ± 8.1</td>
</tr>
<tr>
<td>Transplant LV/blood</td>
<td>2.3 ± 1.9</td>
</tr>
<tr>
<td>Transplant RV/blood</td>
<td>2.5 ± 1.7</td>
</tr>
<tr>
<td>Native LV/blood</td>
<td>0.20 ± 0.2</td>
</tr>
<tr>
<td>Native RV/blood</td>
<td>0.20 ± 0.2</td>
</tr>
</tbody>
</table>

Comparison of ratios of radioactivity in samples of transplant left and right ventricle (LV and RV) with that in native LV and RV and radioactivity in transplant LV, transplant RV, native LV, and native RV with that in blood (n = 15).

activity to the spleen, liver, and bone marrow associated with administration of $^{111}$In-lymphocytes to be 9.0, 1.5, and 0.95 rads/500 µCi, respectively. This compares favorably to the marrow dose of radioactivity in patients treated conventionally for hyperthyroidism with iodine-131 (13 rads).30 In such patients, no increased incidence of leukemia has been detected.31 The FDA has recently approved $^{111}$In-labeled leukocytes for diagnostic studies. A total dose of 500 µCi per annum per patient is presently permitted, sufficient for four to five scintigraphic studies per patient per year for monitoring of potential rejection and guidance of titration of immunosuppressive regimens. A potential algorithm for use of this technique may include alternation of labeled lymphocyte scintigraphy with endomyocardial biopsy in the first few months after transplantation, when rejection is most likely to occur. After this period, when biopsies are performed less frequently, scintigraphy can be used as an alternative. Scintigraphic evidence of mild rejection or clinical suspicion of opportunistic infections would necessitate endomyocardial biopsy. Use of shorter-lived radionuclides would potentially enable more scans to be done annually.

Echocardiographic indexes such as changes in diastolic function are nonspecific or insensitive,32, 33 as are changes in signal-averaged QRS electrocardiographic complexes.34 Magnetic resonance imaging exhibits increased T2 relaxation time with progression of rejection35 and therefore appears promising. However,

**FIGURE 6.** Representative autoradiographs demonstrating labeled lymphocytes in the interstitium of myocardium of an allograft. Rejection was detected scintigraphically and confirmed histologically. (Original magnification × 3000.)
gamma scintigraphy can presently be performed more widely.

The present results indicate that 111In-labeled lymphocyte scintigraphy with blood pool subtraction may provide a sensitive and relatively specific method for noninvasive detection of acute cardiac allograft rejection. This technique merits further evaluation, including assessment of its utility in patients. The approach developed may be particularly helpful for ascertaining the need for and timing of endomyocardial biopsy in individual patients and for monitoring the response of rejection to immunosuppressive drugs.

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