Detection of Cardiac Transplant Rejection with Radiolabeled Lymphocytes

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SUMMARY To determine whether rejection of cardiac transplants could be detected specifically and non-invasively by lymphocytes labeled with indium-111 (111In), we studied 36 allogeneic and 14 isogeneic heterotopic cardiac transplants in rats. Allogeneic grafts accumulated autologous 111In-lymphocytes, detectable scintigraphically 24 hours after i.v. injection of the labeled cells. At the time of peak histologic rejection, the allogeneic grafts accumulated 9.2 ± 4.8 times more activity than the native hearts (determined by well counting). The tissue-to-blood ratio in the rejecting transplants was 3.7 ± 2.2; total uptake by the graft was 2.9 ± 2.1% of the injected dose. Autoradiography confirmed that graft radioactivity was associated with labeled lymphocytes. In contrast, isogeneic grafts showed no signs of rejection and did not accumulate radioactivity. Because conventionally isolated and labeled lymphocytes are often contaminated with platelets, we prepared both 111In-platelets and purified 111In-lymphocytes for use in additional experiments. Allogeneic grafts accumulated platelets and purified lymphocytes independently. Thus, deposition of immunologically active cells in the rejecting graft representing specific pathophysiologic events can be detected. The results suggest that rejection of cardiac transplants can be detected noninvasively, potentially facilitating objective early clinical detection of rejection and titration of antirejection therapy.

EARLY DETECTION of incipient rejection of transplanted organs is an important criterion in titrating antirejection therapy. Clinically, detection is often based on elevated concentrations of macromolecules released into the circulation or into the urine, or on nonspecific manifestations of inflammation, including fever or leukocytosis. In the case of cardiac transplants, invasive endocardial biopsy has been used to increase the sensitivity of detection of rejection crises.

The rejection process has been characterized extensively in experimental animals and in patients. Lymphocytic infiltration is a salient feature. Recently, we and others have used radiolabeled white blood cell elements in experimental animals and patients to identify and localize inflammation accompanying myocardial infarction, myocarditis, or abscess formation. Because methods are now available to facilitate separation and labeling of selected blood cell elements, and because sensitive clinical detection of rejection of cardiac transplants appears likely to become increasingly important, we undertook this study to determine whether sensitive external detection of rejection of cardiac transplants was feasible with lymphocytes labeled with indium-111 (111In).

The rejection process in human kidney transplants is accompanied by accumulation of 111In-labeled platelets. This observation suggests that results of previous studies in which human renal and experimental cardiac transplant rejection was detected after i.v. administration of labeled white cells may have reflected deposition of radiolabeled platelets rather than white cells, because the conventional leukocyte preparations are heavily contaminated with platelets. Because accumulation of lymphocytes is a more specific criterion of rejection and indicates specific pathophysiologic processes, we compared the accumulation of labeled lymphocytes to the less specific deposition of labeled platelets.

We studied rejection of heterotopic cardiac transplants in rats using an experimental design in which each rat could be used as its own control by evaluation of histology and accumulation of radioactivity in both the transplanted and the native heart.

Methods

Animal Preparations

Heterotopic cardiac transplants were performed in rats according to a modification of the technique of Lee et al. and Ono and Lindsey. For studies of allogeneic transplants (n = 119), Wistar-Furth rats (150-250 g, Microbiological Associates) served as donors and Wistar-Lewis (referred to as “Lewis”) rats (175-275 g, Charles River) served as recipients. Wistar-Furth and Lewis rats have a major haplotype incompatibility at the AaB locus. Lewis-to-Lewis transplants (n = 38) served as isogeneic controls.

After induction of anesthesia with diethyl ether, donor rats were subjected to a thoracotomy. The superior vena cava andazygos静脉 were ligated and divided, and the aorta was severed. Before ligation and division of the inferior vena cava, 5-10 ml of modified Krebs-Henseleit buffer were infused into the inferior vena cava. The pulmonary artery was severed and the pulmonary veins were ligated with a pursestring suture. The donor heart was excised rapidly and flushed retrograde through the aorta with 5-10 ml of iced buffer and placed in Krebs-Henseleit buffer at 0-4°C.

Recipient rats were anesthetized similarly and the
abdominal cavity was entered through a midline incision. The abdominal aorta and inferior vena cava were separated and clamped with a Lee portacaval clamp. The donor aorta and pulmonary arteries were then anastomosed in an end-to-side manner to the recipient abdominal aorta and vena cava with 8-0 nylon suture (Davis and Geck) (fig. 1). Accordingly, as soon as the clamp was released, the donor heart was perfused retrograde through the aorta. The operation, from incision of the donor to closure of the recipient’s abdominal cavity, lasted 25-40 minutes.

Function of the transplant was assessed daily by palpation to detect beating frequency and the gross quality of contraction. In addition, on selected days after transplantation, electrocardiographic activity of the transplanted heart was assessed by anesthetizing the rat and placing a standard precordial lead over the point of maximal impulse of the heterotopic transplant. Interference from the rat’s own heart was minimized by placing the arm leads at the subcostal margin. Complete rejection was defined as cessation of both palpable contractions and electrical activity.

Histology and Autoradiography

At the time of graft rejection or elective sacrifice, rats were anesthetized with ether and the transplanted heart was excised. Hearts were cut transversely at 3-mm intervals and fixed in calcium-acetate-buffered 10% formalin. Paraffin-embedded tissue was sectioned and stained with hematoxylin-eosin. Selected sections were examined with Masson’s trichrome, Verhoeff-Van Gieson’s, phosphotungstic acid hematoxylin, and pyronin stains. All hearts were evaluated in a blinded fashion by one of the authors.

Autoradiographs were prepared by applying paraffin sections to gelatin-coated slides. The tissue was deparaffinized in toluene and dried. The slides were 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 thoroughly dried in the vertical position, placed in light-tight plastic boxes containing silica gel desiccant, and stored at 4°C. After 3-14 days, the slides were warmed to 20°C and processed sequentially in Kodak D-19 developer, distilled water and fixer; the slides were then rinsed three times with distilled water and stained with hematoxylin-eosin.

Labeling of Blood Cell Elements

For conventional lymphocyte separation and labeling, 1.0 ml of blood was withdrawn from the tail of the recipient and anticoagulated with acid-citrate-dextrose (ACD). Rat blood collected in this fashion contains 6000-20,000 leukocytes/mm³, of which approximately 80% are lymphocytes.¹⁷ The blood was centrifuged in 75 × 12-mm polycarbonate tubes for 5 minutes. All centrifugations were performed at 360 g at 22°C unless otherwise noted. The leukocyte-rich plasma was removed along with the top layer of red blood cells containing settled white blood cells, and 500 µl of 0.9% NaCl-ACD (7:1 vol/vol) was added and mixed gently. The suspension was layered onto

**Figure 1. Diagram of the rat heterotopic cardiac transplant preparation.**
1.5 ml of Ficoll-Paque (Pharmacia) and centrifuged for 30 minutes. The lymphocyte layer was removed and washed twice with NaCl-ACD by centrifugations at 600 g for 10 minutes. Recovery of lymphocytes was typically 30%.

Lymphocytes prepared in this conventional fashion are contaminated with approximately 10–20 platelets per lymphocyte, based on hemocytometry. Accordingly, a more purified lymphocyte preparation was developed and evaluated. Anticoagulated blood (1.5 ml) was centrifuged for 10 minutes; the plasma was removed and the buffy coat and the top one-third of erythrocytes (containing settled lymphocytes) were aspirated and mixed with 200 µl of NaCl-ACD. This mixture was layered on top of 1.5 ml of Ficoll-Paque and centrifuged for 30 minutes. The lymphocyte layer was aspirated and washed twice with NaCl-ACD and centrifuged at 600 g. The pellet was diluted with 200 µl of NaCl-ACD and 200 µl of platelet-free plasma layered on 1.5 ml Ficoll-Paque and centrifuged once more. The lymphocytes were then washed twice by centrifugation with NaCl-ACD, and subsequently washed twice in 1 ml of platelet-free plasma with centrifugations for 1.5 minutes before a final wash in NaCl-ACD by centrifugation at 600 g. The additional separations with Ficoll-Paque and washes with plasma reduced platelet contamination in each case to a maximum of one platelet per lymphocyte.

To label the harvested lymphocytes, a modification of the method of Thakur et al.9 was used. Two hundred microliters NaCl-ACD were added to 1.25 µl of 111In-oxine (obtained from Diagnostic Isotopes as 1 mCi in 50 µg of oxine and 50 µl of ethanol) and added slowly to the cell pellet containing 3–9 × 10⁶ lymphocytes. Cells were incubated for 20 minutes at room temperature, centrifuged at 600 g for 10 minutes, resuspended in 200 µl of the platelet-free plasma to remove free 111In by binding to transferrin, centrifuged, and resuspended in 400 µl of NaCl. The labeling efficiency was 35–50%.

Since radioactivity, oxine and ethanol can each be toxic to lymphocytes under specific conditions,18–20 viability of the labeled cells was assayed on three occasions with a nigrosin dye exclusion technique.21 Based on results with this procedure, 97% or more of the lymphocytes were viable after the separation and labeling procedure, corroborating reports using similar methods.18, 22 To exclude artifacts potentially attributable to loss of cell function due to labeling, some studies were performed with only 10% or 1% of the usual amount of radioactivity, oxine or ethanol by first allowing the tracer to decay or by appropriate dilutions of reagents.

For studies with platelets, labeling was performed after centrifugation of 1.5 ml of ACD-anticoagulated blood for 10 minutes. The platelet-rich plasma was aspirated and centrifuged at 2800 g for 15 minutes and washed twice with NaCl-ACD by centrifugation at 2800 g for 15 minutes. Evaluation of smears with Wright's stain showed that the platelet pellet contained no lymphocytes. Platelets were labeled with 111In-oxine with the same method used to label lymphocytes.23 The labeled platelets were washed once with plasma and then suspended in 400 µl of NaCl.

Tracer that might have been released from labeled cells in vivo would become bound to transferrin. Accordingly, control studies were performed in which the accumulation of exogeneous, labeled transferrin was quantified in rejecting hearts. Plasma transferrin was labeled in vivo after i.v. injection of 10 µCi of 111In-chloride (Medi-Physics). Under these conditions, indium injected in the absence of oxine binds avidly to transferrin but does not label cells. All labeled components were injected intravenously after the rats had been anesthetized at selected intervals after transplantation.

Detection of Radioactivity
Twenty-four hours after injection of radioactivity, rats were anesthetized and placed in a supine position. Anteroposterior scintigraphs were obtained with a Medex X updated Nuclear Chicago Pho-Gamma III scintillation camera with a 4000-parallel-hole, medium-energy collimator, set for both major gamma energy peaks of 111In (171 and 247 keV). A total of 10,000 counts was collected. Immediately after gamma scintigraphy, the rats were sacrificed and radioactivity in selected organs was assayed with a MicroMedics gamma well counter. In addition, blood components were separated and radioactivity associated with specific blood fractions was quantitated.

Experimental Protocol
One hundred nineteen allogeneic and 38 isogeneic transplants were performed (table 1). Rats in which the transplanted heart failed to initiate contraction or stopped beating within 72 hours after transplantation because of ischemic damage (documented histologically and readily differentiated from rejection) were considered technical failures and excluded from the study.

To define the temporal characteristics of the preparation, 40 of the 86 technically successful allogeneic transplants were maintained until complete rejection occurred. Forty-six grafts were selected for

<table>
<thead>
<tr>
<th>Table 1. Summary of 157 Heterotopic Cardiac Transplants in Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isogeneic group (Lewis-to-Lewis transplants)</td>
</tr>
<tr>
<td>Total operated</td>
</tr>
<tr>
<td>Technically successful</td>
</tr>
<tr>
<td>Labeling experiments</td>
</tr>
<tr>
<td>Elective sacrifice</td>
</tr>
<tr>
<td>Technical failures</td>
</tr>
<tr>
<td>Allogeneic group (Wistar-Furth-to-Lewis transplants)</td>
</tr>
<tr>
<td>Total operated</td>
</tr>
<tr>
<td>Technically successful</td>
</tr>
<tr>
<td>Complete rejection</td>
</tr>
<tr>
<td>Labeling experiments</td>
</tr>
<tr>
<td>Technical failures</td>
</tr>
</tbody>
</table>

Technical failures included hearts that lacked initiation or sustenance of contractile and electrical activity within 72 hours of surgery.
TABLE 2. Comparison of the Accumulation of Conventionally Prepared 111In-labeled Lymphocytes by Allogeneic and Isogeneic Heterotopic Cardiac Transplants at Selected Intervals After Transplantation

<table>
<thead>
<tr>
<th>Days after transplant</th>
<th>n</th>
<th>Hr/Ho</th>
<th>Hr/BLD</th>
<th>Uptake (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Isogeneic group</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-4</td>
<td>3</td>
<td>1.8 ± 1.0</td>
<td>0.3 ± 0.2</td>
<td>0.4 ± 0.3</td>
</tr>
<tr>
<td>5-6</td>
<td>5</td>
<td>1.3 ± 0.8†</td>
<td>0.6 ± 0.5</td>
<td>0.5 ± 0.3</td>
</tr>
<tr>
<td>7-8</td>
<td>6</td>
<td>1.1 ± 0.4†</td>
<td>0.4 ± 0.5†</td>
<td>0.4 ± 0.1†</td>
</tr>
<tr>
<td><strong>Allogeneic group</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-4</td>
<td>3</td>
<td>1.4 ± 0.9</td>
<td>0.3 ± 0.2</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>5-6</td>
<td>11</td>
<td>6.5 ± 2.6*†</td>
<td>1.2 ± 1.0</td>
<td>3.1 ± 2.3*</td>
</tr>
<tr>
<td>7-8</td>
<td>10</td>
<td>9.2 ± 4.8*†</td>
<td>3.7 ± 2.2*†</td>
<td>2.9 ± 2.1*†</td>
</tr>
</tbody>
</table>

All ratios are normalized per gram tissue. Uptake refers to the total radioactive uptake of the Hr as a percentage of injected radioactivity. Values are mean ± SD.

*p < 0.05 vs days 3-4 (within group).
†p < 0.05 vs days 3-4 (between groups).

Abbreviations: Hr = transplanted heart; Ho = rat's own heart; BLD = blood.

Labeling experiments. Twenty-four of these rats were injected with conventionally prepared 111In-lymphocytes during one of three intervals: days 2–3, 4–5 or 6–7. Twenty-four hours after injection, scintigraphy was performed and the hearts were removed. Radioactivity was determined with a well-counter (table 2).

Of the 23 successful isogeneic transplants, nine were sacrificed at days 3–188 for histologic evaluation (table 1). Fourteen rats were injected with conventionally prepared 111In-lymphocytes during corresponding intervals after transplantation and imaged 24 hours after injection (table 2).

Twelve additional rats with allogeneic grafts received purified 111In-lymphocytes, five received 111In-platelets and three received 111InCl (to label transferrin) 7 days after transplantation and imaged 24 hours after injection (table 3). Two additional rats with 7-day allografts received lymphocytes labeled with 10% of the generally used amounts of radioactivity, oxine or ethanol.

Statistical Analysis

Comparisons between allogeneic and isogeneic groups or between allogeneic transplants labeled with different blood components were performed with a modified t test for independent samples, incorporating the Bonferroni correction for multiple comparisons, after analysis of variance.24

Results

One hundred nine technically successful heterotopic cardiac transplantations were performed. Of these, 86 were allogeneic transplants and 23 were isogeneic transplants. Forty of the allogeneic transplants were maintained until complete rejection occurred, defined as the complete cessation of palpable contractions and cessation of electrical activity of the donor heart. The mean interval to complete rejection was 7.5 ± 1.8 (±SD) days after transplantation. An additional 46 rats with allogeneic grafts were sacrificed at selected intervals after transplantation for histologic analysis and for determination of accumulation of radiolabeled components.

The isogeneic group comprised 23 transplants. No transplanted heart in this group exhibited the criteria of rejection. All transplanted hearts were beating well at the time of elective sacrifice and exhibited normal electrocardiographic activity.

Electrocardiographically, all technically successful isogeneic and allogeneic grafts exhibited normal sinus rhythm 1 day after surgery. Heart rate and electrocardiographic wave-form amplitude and duration did not change in isogeneic grafts throughout the entire study. In contrast, as reported previously,3,4,14 electrical activity from allogeneic grafts reflected progressive slowing of heart rate, diminished QRS amplitude, and widened QRS complexes with eventual complete cessation of electrical activity.

All isogeneic grafts beat forcefully until the time of elective sacrifice, 3–188 days after transplantation. In contrast, allogeneic grafts became progressively less compliant, exhibited progressively less forceful contractile function and eventually became noncontractile.

Sequential Histologic Changes

Allogeneic and isogeneic grafts were indistinguishable during the first 48 hours after surgery. Both showed acute epicarditis and mild interstitial edema consistent with surgical handling and disruption of cardiac lymphatics. In isogeneic grafts, these changes gradually resolved. Within 7–10 days the myocardium had a normal histologic appearance and the epicardium was covered with bland fibrous tissue (fig. 2). The myocardium remained histologically normal in isogeneic grafts examined up to 188 days after surgery.

In contrast, allografts became infiltrated by mononuclear cells by the third day after surgery. The infiltrate accumulated rapidly and was extremely dense by the fourth to fifth day. Most of the cells were large lymphocytes containing vesicular nuclei and pyrinnophilic (RNA-rich) cytoplasm. The later stages of re-

Table 3. Comparison of the Accumulation of Selectively Prepared Blood Components Labeled with Indium-111 by 8-day Allogeneic Grafts

<table>
<thead>
<tr>
<th>Cell preparations labeled with 111In</th>
<th>n</th>
<th>Hr/Ho</th>
<th>Hr/BLD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventionally prepared lymphocytes</td>
<td>10</td>
<td>9.2 ± 4.8</td>
<td>3.7 ± 2.2</td>
</tr>
<tr>
<td>Purified lymphocytes</td>
<td>12</td>
<td>7.0 ± 2.4</td>
<td>1.8 ± 0.5*</td>
</tr>
<tr>
<td>Platelets</td>
<td>5</td>
<td>7.2 ± 2.8</td>
<td>2.9 ± 2.3</td>
</tr>
<tr>
<td>Transferrin</td>
<td>3</td>
<td>1.8 ± 0.9*</td>
<td>0.3 ± 0.1*</td>
</tr>
</tbody>
</table>

Values are mean ± SD.

*p < 0.05 vs results with conventionally prepared lymphocytes.

Abbreviations: See table 2.
Accumulation of Radioactivity by Transplanted Hearts

Thirty-eight rats with cardiac transplants were injected with lymphocytes labeled with the conventional preparation described in the Methods section (table 2). All were studied by scintigraphy and sacrificed 24 hours after injection of labeled cells. All transplanted hearts accumulated some radioactivity 3–4 days after transplantation, probably due to the surgically related inflammation recognized histologically (fig. 3). However, in isogeneic transplants, accumulation diminished subsequently and was virtually nil within 4–7 days. In contrast, allogeneic cardiac transplants accumulated label progressively with time. By day 5–6 after transplantation (in rats with cells administered during the day 4–5 interval), when allografts were functionally unimpaired as assessed by palpation and electrocardiography, increased accumulation of labeled cells in the transplanted heart was readily detectable scintigraphically (fig. 3) and by the results of well counting (table 2). In all rats, liver and spleen activity was prominent due to slow transit and cellular accumulation of lymphocytes by the spleen and due to trapping of immunologically competent and damaged labeled cells by the reticuloendothelial system. In 7–8-day-old allogeneic transplants, the transplanted heart accumulated an average of 2.9% of the injected dose of radioactivity and the
tissue-to-blood ratio averaged 3.7. Rejecting allogeneic grafts were prominent scintigraphically (fig. 3). In contrast, 7-day-old isografts accumulated only 0.4% of the injected dose and the tissue-to-blood ratio was consistently less than 0.5 (table 2).

All scintigraphs were read by two independent observers who had knowledge of the technique, but were blinded with respect to individual protocols. All scintigraphs from rats with 3–4-day grafts or with 7–8-day grafts were interpreted correctly for allogeneity or isogeneity by both observers, suggesting reliable specificity and sensitivity of the technique. Scintigraphs from 5–6-day-old isografts were all correctly identified as negative, and observers agreed on the positivity or negativity of rejection in 5–6-day-old allografts corresponding to the individual natural variability of the immune response, corroborated by histologic examination of individual specimens.

Because conventional lymphocyte preparations can be extensively contaminated with platelets, additional studies were performed. Twelve rats with 7-day-old allogeneic grafts were injected with a purified suspension of 111In-labeled lymphocytes prepared as described in the Methods section. Five additional rats with 7-day-old transplants were injected with 111In-labeled platelets and three rats with 7-day-old cardiac transplants were injected with 111InCl₃ to label plasma transferrin. Both purified labeled lymphocytes and labeled platelets accumulated in the transplanted allografts in concentrations approximately seven times greater than those in the rat’s own heart and to an extent similar to that observed in rats with 7–8-day allografts injected with labeled lymphocytes prepared conventionally (table 3). Presumably because of inflammatory changes and altered capillary permeability in the rejecting organ, leakage of labeled transferrin from the blood into the transplanted organ was manifest by increased activity after i.v. injection of 111InCl₃ (labeling plasma transferrin) in allografts at day 7, but to a much smaller extent than that seen with either lymphocytes or platelets (table 3). Thus, it appears that both labeled lymphocytes and labeled platelets accumulate in rejecting allografts and that both contribute to radioactivity observed after injection of platelet-contaminated, conventionally prepared lymphocytes.

Autoradiographs from transplanted hearts obtained 8 days after surgery indicated that after administration of the purified lymphocyte preparation, accumulation of radioactivity in allografts was clearly attributable to labeled lymphocytes in the rejecting heart (fig. 4). Autoradiographs from a 7-day allograft injected with 111In-labeled platelets showed that activity incorporated into the heart was due to labeled platelets, which exhibit grain patterns markedly different from those of labeled lymphocytes.

**Discussion**

Indium-111 is a clinically attractive tracer because it has a relatively short half-life (2.8 days) and a suitable energy spectrum, with a consequent low radiation burden. This isotope has been used in clinical studies involving labeling of a wide variety of cells and detection of processes such as myocardial infarction,6–7 abscess formation,8 thrombosis,9 and lymphoproliferative disorders.10 These successful applications suggest that leukocytes and platelets labeled with 111In retain their functional properties, even though lymphocytes may be more radiosensitive than many other cells.6–13, 18–20, 22, 23, 25–29 As currently used clinically, the radiation burden of 111In is low, and as little as 150 μCi affords adequate scintigraphic definition.10 Thakur et al.28 calculated that for a 1-mCi injection of 111In-leukocytes, the maximal radiation burden would be 6–18 rads to the spleen, 1–5 rads to the liver, and approximately 0.5 rads whole body dose.29

The results of the present study indicate that 111In-labeled lymphocytes accumulate in rejecting grafts. This accumulation can be detected as early as 5 days after transplantation, when marked mechanical, functional and electrocardiographic signs of rejection are not apparent (table 2, fig. 3).

The histologic changes seen in allografts are compatible with previous reports and correlate well with
cardium. This immunologic rejection was heralded by lymphocytic infiltration, and when rats with allogeneic transplants were injected with autologous $^{111}$In-labeled lymphocytes, the grafts accumulated activity, permitting scintigraphic identification. Autoradiographs obtained from allografts after administration of the purified lymphocyte preparation showed that the activity was associated with lymphocytes and confirmed our impression that the labeling technique permits specific detection of infiltration of lymphocytes in rejecting hearts (fig. 4).

The hepatic and splenic radioactivity observed (table 4) reflects participation of these organs in the physiological fate of lymphocytes and also in the removal of damaged cells from the circulation. Thus, some of the activity may reflect removal of lymphocytes injured by isolation or by labeling. In autoradiographs of liver, grains were found dispersed over sinusoids and clustered over Kupfer cells (fig. 5). Intact labeled lymphocytes were not present in the liver, which suggests that hepatic radioactivity was derived from damaged cells or noncellular labeled material removed by the reticuloendothelial cells. Autoradiographs of the heavily labeled spleen indicated that the majority of activity was associated with intact lymphocytes in the lymphoid aggregates (white pulp) surrounding splenic arterioles. Presumably, the labeled lymphocytes in white pulp were viable cells trafficking through the spleen either nonspecifically or as part of the specific immune response to the allograft. The relatively low levels of radioactivity in splenic red pulp and liver compared with splenic white pulp suggests that accumulation of label due to scavenging of damaged cells or noncellular elements is minimal in these organs.

**TABLE 4. Percent Uptake of Injected Activity in Selected Organs 24 Hours After Injection of Indium-111-labeled Blood Components**

<table>
<thead>
<tr>
<th>Cell preparation (n)</th>
<th>Lung</th>
<th>Spleen</th>
<th>Liver</th>
<th>Kidney</th>
<th>Blood</th>
<th>Plasma/blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventionally prepared lymphocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allogeneic (10)</td>
<td>1.8 ± 0.6</td>
<td>8.6 ± 2.4</td>
<td>15.9 ± 5.0</td>
<td>0.9 ± 0.3</td>
<td>1.2 ± 0.4</td>
<td>3.5 ± 2.0</td>
</tr>
<tr>
<td>Isogeneic (5)</td>
<td>1.7 ± 0.5</td>
<td>12.6 ± 5.6</td>
<td>17.8 ± 8.3</td>
<td>1.0 ± 0.1</td>
<td>1.3 ± 0.6</td>
<td>5.9 ± 3.2</td>
</tr>
<tr>
<td>Purified lymphocytes (12)</td>
<td>1.0 ± 0.6</td>
<td>10.4 ± 1.0</td>
<td>21.0 ± 7.1</td>
<td>0.9 ± 0.1</td>
<td>0.3 ± 0.08</td>
<td>8.9 ± 5.2</td>
</tr>
<tr>
<td>Platelets (5)</td>
<td>1.3 ± 0.5</td>
<td>9.6 ± 2.5</td>
<td>15.1 ± 1.5</td>
<td>0.8 ± 0.3</td>
<td>1.0 ± 0.4</td>
<td>3.9 ± 1.7</td>
</tr>
<tr>
<td>Transferrin (3)</td>
<td>1.0 ± 0.4</td>
<td>1.0 ± 0.1</td>
<td>24.6 ± 15.9</td>
<td>5.0 ± 2.3</td>
<td>0.9 ± 0.8</td>
<td>70.0 ± 10.3</td>
</tr>
</tbody>
</table>

The values (mean ± sd) indicate the absolute percentage of activity in the whole organ (corrected for isotope decay), except for blood uptake, which represents the percentage of injected activity in 1 ml of blood 24 hours after injection. The plasma/blood ratio indicates the amount of label released from cells that binds to plasma transferrin.
In the present study, lymphocyte viability after labeling was ascertained by a dye-exclusion procedure, which indicated greater than 97% viability. Further, the presence of intact lymphocytes in the spleen indicates normal lymphocyte function, as noted by others.18, 20, 22, 26, 27 The results of additional experiments (data not shown) indicated that labeling lymphocytes with 1% or 10% the amount of radioactivity, oxine or ethanol led to identical distributions of lymphocytes (as a percentage of injected cells) in organs and in rejecting hearts. This amount of activity did not permit noninvasive imaging. Others have found that damaged cells accumulate in the lungs.9, 18 In the present study, lung radioactivity was negligible, supporting the impression that lymphocyte viability was well preserved.

In rats, the small blood volume limits the number of autologous lymphocytes that can be isolated and labeled. Because the efficiency of the labeling is related not only to radioactivity per se but also to the number of cells used,9, 13, 19, 22 it is likely that for clinical purposes, isolation and labeling efficiency would be higher, and that potential damage to lymphocytes could be reduced even further. In addition, appropriate shielding and subtraction of activity emanating from the spleen and liver would improve image resolution and increase the likelihood of detecting rejection early.

Clinical Implications

The results of this study indicate that organ rejection can be detected with 111In-labeled lymphocytes. Although conventional lymphocyte preparations are heavily contaminated with platelets and although 111In-labeled platelets accumulate in rejecting hearts presumably secondarily to vascular injury that occurs concurrently with rejection, specific detection of the inflammatory response characteristic of the rejection process is possible with the use of a purified lymphocyte preparation. Deposition of labeled cells is independent of deposition of 111In-transferrin, which also accumulates in rejecting hearts, albeit to a much smaller extent. Thus, with the use of selected blood components, all of which can be labeled with 111In, specific pathophysiologic aspects of rejection can be identified. Accumulation of labeled lymphocytes heralds the onset of immunologic rejection. We recently reported that 111In-lymphocytes accumulation is suppressed in rats in which allograft rejection is mitigated by immunosuppressive therapy.60 Thus, detection of such accumulation is likely to provide a relatively specific and sensitive criterion for detecting incipient rejection and may improve objective determination of the efficacy of antirejection therapy.

Addendum


Acknowledgment

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