Cellular Basis for Accumulation of In-111-Labeled Leukocytes and Platelets in Rejecting Cardiac Allografts: Concise Communication

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Biodistribution and imaging studies in rats showed that In-111-labeled leukocytes and platelets accumulate progressively with time after transplantation in cardiac allografts undergoing rejection, but do not accumulate in normal syngeneic heart grafts. Maximum heart allograft-to-blood ratios of 9:1 were obtained, and allograft-to-native heart ratios of 17:1. Microscopic studies of the rejecting cardiac allografts showed that histologic findings paralleled the cellular changes predicted by the radionuclide studies. Intravenously administered Ga-67 citrate and Tc-99m sulfur colloid failed to show significant accumulation in rejecting grafts. The findings suggest that cellular rejection, rather than nonspecific inflammatory changes, is the primary basis for accumulation of In-111 leukocytes and platelets in rejecting cardiac allografts.

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A number of centers throughout the world perform cardiac transplantation to treat certain forms of end-stage heart disease. Current techniques for the diagnosis of cardiac allograft rejection include electrocardiography (which may be unreliable), endomyocardial biopsy (which cannot be performed frequently), and immunological monitoring (*1*). A number of studies (*2–5*) have shown that leukocytes are essential for allograft rejection, and that these cells infiltrate the rejecting graft before the onset of clinical rejection (*6*). Recent reports indicate that both Ga-67 (*7*) and In-111 (*8*) can be monitored externally when incorporated into leukocytes. The use of Ga-67 is limited because only 6% of its activity is incorporated in vitro with leukocytes. However, 80–95% leukocyte labeling efficiencies can be achieved using In-111 oxine (*8,9*). Indium-111 is suitable for cell labeling and tracing biological processes in vivo because it has a half-life of 67 hr and gamma emissions that permit imaging up to a week after infusion. Indium-111-labeled leukocytes have been used to detect abscesses (*8,11,12*), renal allograft rejection (*13*), and the inflammatory response associated with myocardial ischemia (*14*). Indium-111-labeled platelets have been utilized for evaluation of vascular thrombogenesis (*15,16*) and renal allograft rejection (*17*). We have recently shown that in the rat In-111-labeled leukocytes and platelets, together with external gamma imaging, can detect acute cardiac allograft rejection in vivo (*18*).

The purpose of the present study was to investigate the cause of accumulation of In-111-labeled cells in rejecting cardiac allografts, primarily by correlating the radionuclide accumulation with histological studies of the rejecting graft.

MATERIALS AND METHODS

Adult Lewis (RT-11) rats* served as recipients of heterotopic cardiac allografts from either ACI (RT-1a) rats* or syngeneic cardiac grafts from Lewis rats. The donor and recipient rats were anesthetized with ether and intraperitoneal chloral hydrate. The donor heart was removed and transplanted to the peritoneal cavity of the recipient using the technique of Ono and Lindsay (*19*). In this procedure the donor aorta and pulmonary artery are anastomosed end-to-side to the recipient's abdominal aorta and vena cava, respectively. Technical failures with

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this technique occur in less than 5% of animals. Viability of the heart allograft was determined by daily palpation. All transplanted hearts were still beating strongly at the time of sacrifice.

Sterile, heparinized blood was obtained for cell labeling from control Lewis rats. Blood was pooled from several such animals so that an adequate volume (≥30 ml) could be obtained for labeling. Leukocytes were separated by Ficoll-Hypaque fractionation for the separation of small blood volumes, modified from the technique described by Boyum (*20*). The leukocytes (≥92% lymphocytes) were suspended in 2 cc of normal saline and incubated at room temperature for 15 min with In-111 oxine† as described by Thakur et al (*8*). The cells were washed twice with normal saline. Counts of In-111 in the washings and cell plug consistently showed that 80–95% of the activity was carried in the cell fraction. The labeled cells were then resuspended in normal saline at a concentration of 2×10^8 cells/ml. The viability of these labeled cells was over 95% as determined by trypan blue dye exclusion.

Platelets were separated from whole-blood samples obtained from Lewis rats by the technique of Scheffel et al (*21*). The platelet suspension in triple-phosphate solution was incubated with In-111 oxine at 37°C in a water bath for 10 min (*22*). The cells were washed in triple-phosphate sodium twice, then resuspended at a concentration of 2×10^8 cells/ml. The platelet labeling efficiency was 35–50%.

Lewis recipients of ACI allografts or syngeneic grafts were divided into five groups. Group 1 (n = 50) received intravenously 2×10^7 leukocytes labeled with 15–20 μCi of In-111, and Group 2 (n = 36) received 2×10^7 platelets labeled with the same amount of In-111. Groups 3 (n = 18) and 4 (n = 21) were controls that received Ga-67 citrate (50 μCi) and Tc-99m sulfur colloid (100 μCi), respectively. These control studies were performed to assess the impact of nonspecific factors on radionuclide accumulation in the transplanted heart. The Ga-67 control was used to assess the role of nonspecific inflammation, and the Tc-99m sulfur colloid control to assess the importance of nonspecific particle trapping (e.g., fibrin entrapment) in the transplanted heart. Both controls were also used to indicate what amounts of activity could be expected to accumulate in the transplant secondary to nonspecific factors like fluid extravasation. In Group 5, 20 rats were injected with labeled cells pooled from Lewis rats that had been transplanted three days earlier with ACI cardiac allografts. This study was performed to determine whether the behavior of labeled cells was different after they had been "sensitized" by the presence of an allograft. These experiments were performed with In-111 leukocytes.

Labeled cells were injected each day after transplantation into 4–7 animals in each group, and the animals were studied 24 hr later. Rats receiving Ga-67 were studied 24 hr after injection, but animals that received Tc-99m sulfur colloid were studied on the same day, approximately 4 hr after injection. Transplanted animals (n = 4–7 per group) and controls (n = 3 per group) were killed on days 1–7 after transplantation. Tissues were excised, weighed, and counted for 10 min in a well scintillation counter. Count ratios (e.g., heart to blood, transplanted heart to native heart, etc.) were determined in terms of percent injected dose per g for each animal, and expressed as the mean ratio for each group.

Syngeneic and allogeneic cardiac allografts were also examined by light microscopy from post-transplant days 1–7 (n = 2 per period). The resected hearts were fixed in 10% neutral formalin solution and stained by hematoxylin-eosin and periodic acid Schiff reagent for histology. Grading of cellular infiltration was performed by a pathologist who had no knowledge of the imaging or tissue-counting experiments. The following grading scheme was used: 0 = no cellular infiltration; + 1 = mild, +2 = moderate, and +3 = marked cellular infiltration. Histologic changes were used as the standard for determining the presence and degree of rejection.

RESULTS

Figure 1 summarizes the accumulation patterns of the different radionuclides in the heart allografts. Indium-111-labeled leukocytes showed gradual accumulation in the heterotopic cardiac allografts from days 1–3. Thereafter there was a mounting accumulation of labeled leukocytes in the allograft. This trend continued until the time of rejection. The accumulation of leukocytes in the graft on day 3 was significantly greater (p

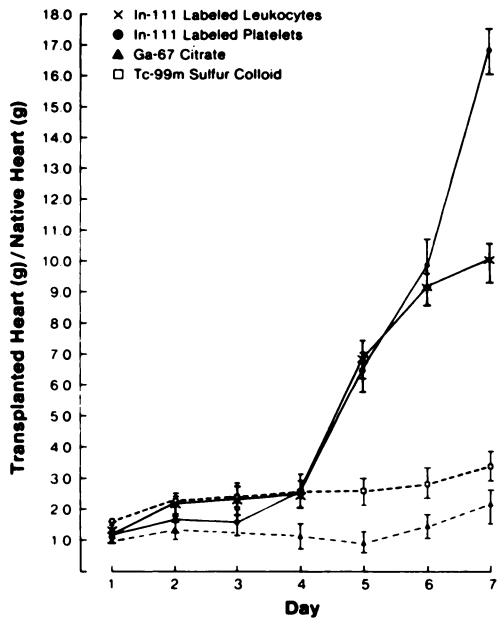

FIG. 1. Ratio of concentration of radioactivity in heterotopic cardiac allografts relative to native hearts.

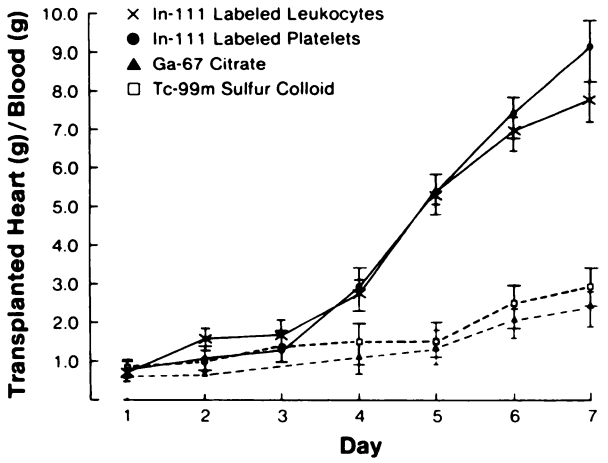

FIG. 2. Ratio of concentration of radioactivity in heterotopic cardiac allografts relative to blood.

<0.001) than in syngeneic grafts and native hearts (*18*).

The activity in cardiac allografts, relative to blood, also showed a significant and progressive rise (Fig. 2). At day 5 the concentration of In-111 in the allografted heart was 6.3 times that in the native heart, and 5.6 times that in the blood. Results in animals that received In-111 leukocytes from "sensitized" animals were not significantly different from those obtained with blood pooled from controls (Table 1).

Heterotopic cardiac allografts showed a gradual accumulation of labeled platelets from days 1–4. On day 5 there was a sharp rise in activity. At this time the platelet activity in allografts (% injected dose/g) was nearly 7 times that in native hearts and syngeneic grafts, and nearly seven times that in the allografts on day one. On post-transplant day 7, the last ratio was nearly 17:1 (Fig. 1), and similar marked increases in the allograft-to-blood ratios occurred (Fig. 2). Syngeneic grafts did not show significant accumulation of labeled platelets during the course of the study.

Biodistribution studies (Figs. 1, 2) showed no significant increase in Ga-67 activity in the transplanted allografts relative to the native hearts or blood during the course of the study. Tc-99m sulfur colloid did show a small but statistically significant increase in allograft activity (Fig. 1). There were no significant differences in Ga-67 or Tc-99m sulfur colloid accumulation in allografts, compared with syngeneic grafts. These findings suggest that, in this study, nonspecific factors like capillary permeability or fibrin trapping may have made a small contribution to the mechanisms of tracer accumulation. However, this could not account for the marked increases in uptake seen with In-111-labeled platelets or leukocytes.

Serial histologic studies of rejecting cardiac allografts showed that focal areas of myocardial infiltration by small numbers of lymphocytes and monocytes were first noted at 24 hr after transplantation (Fig. 3). The epicardium, myocardium, and endocardium all demonstrated increased mononuclear cellular infiltration—mainly lymphocytes, monocytes, and macrophages. This trend toward mononuclear cell infiltration continued until rejection occurred between day 4 and day 6. Focal areas of myocardial necrosis, infiltration, and interstitial hemorrhage were first seen on day 4, and these progressed to large necrotic areas that were heavily infiltrated by neutrophils by day 5. This trend continued until rejection. The coronary and myocardial blood vessels first showed evidence of intraluminal thrombi and platelet aggregation at day 5. This trend continued until rejection. The syngeneic grafts showed infiltration of the epicardium by a few of neutrophils and mononuclear cells at sites of suture. Cellular infiltration at the suture site subsequently became less noticeable.

Comparison of cardiac allograft cell infiltration, determined histologically, with that calculated from tissue concentration of radioactivity in the same animals, demonstrated close agreement for both In-111-labeled leukocytes (Fig. 4) and In-111-labeled platelets (Fig. 5).

TABLE I. CARDIAC UPTAKE OF In-111-LABELED LEUKOCYTES FROM SENSITIZED VS. CONTROL DONORS*

Transplant day	(N)	Transplanted heart / native heart ratio† Control (unsensitized)	Transplanted heart / native heart ratio† Control (sensitized)	Transplanted heart / blood ratio† Control (unsensitized)	Transplanted heart / blood ratio† Control (sensitized)
1	5	1.26 ± 0.10	2.31 ± 0.24	0.64 ± 0.10	0.65 ± 0.11
3	5	2.30 ± 0.21	2.50 ± 0.18	1.77 ± 0.21	1.82 ± 0.24
5	5	6.94 ± 0.63	6.42 ± 0.78	5.64 ± 0.74	5.34 ± 0.70
7	5	10.07 ± 0.84	9.79 ± 0.45	7.91 ± 0.92	7.38 ± 0.84

* Data presented as mean ratio ±1 s.d. Differences are nonsignificant for all data pairs.

† Calculated for mean % dose/g of transplanted heart ÷ mean % dose/g of native heart or blood.

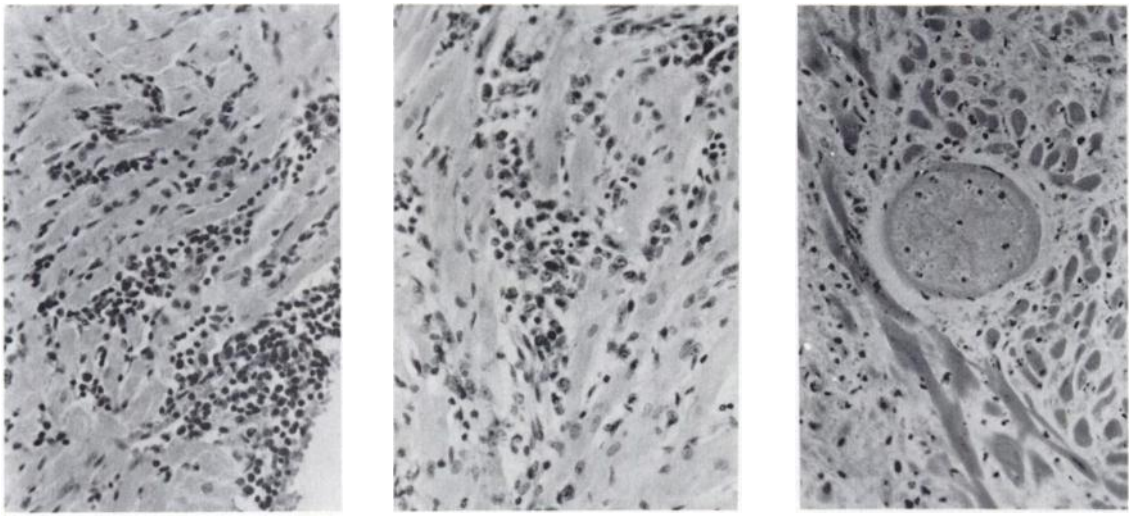

FIG. 3. Cardiac allograft histology. At 2 days after transplantation there is predominantly a mononuclear cell infiltrate (left). At 4 days after transplantation there is infiltration with both mononuclear cells and neutrophils (center). At 6 days after transplantation there is evidence of vessel thrombosis and cellular necrosis (right). H & E X 300.

DISCUSSION

Indium-111-labeled leukocytes have been shown to be effective in the detection of abscesses in experimental animals (*8,12*) and in man (*11*). In addition, In-111-labeled leukocytes have been shown to accumulate in regions of inflammation secondary to cardiac ischemia (*14*) and during renal allograft rejection (*13*). The present study confirms that In-111-labeled leukocytes are useful in the detection of rejecting cardiac allografts. This finding is indirectly predicted by the fact that a variety of cells, including granulocytes, monocytes, lymphocytes, and macrophages, are present during rejection of allografts (*2-6*) and correlates with the histologic presence of these cells in rejecting allografts in the current study.

Our data show that labeled leukocytes infiltrate the rejecting cardiac allografts before clinical rejection at a time when the hearts are still beating strongly. Indium-111-labeled leukocytes and platelets accumulate progressively with time after transplantation in cardiac allografts, but not in heterotopic syngeneic cardiac grafts. The failure of Ga-67 citrate or Tc-99m sulfur colloid to show substantial amounts of accumulation suggests that nonspecific factors—such as tissue extravasation, infection, bleeding at the site of the graft, or trapping during fibrin deposition—are not major

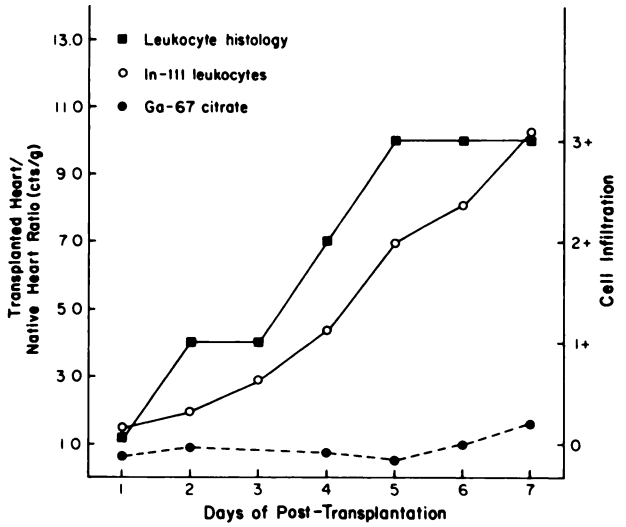

FIG. 4. Relationship between cellular infiltration in cardiac allografts, as determined histologically, and that calculated from tissue radioactivity concentration due to In-111-labeled leukocytes.

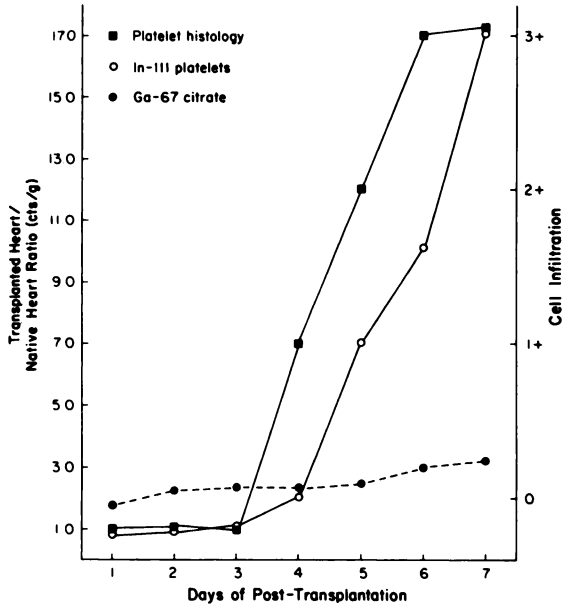

FIG. 5. Relationship between cellular infiltration in cardiac allografts, as determined histologically, and that calculated from tissue concentration of radioactivity due to In-111-labeled platelets.

factors in the accumulation of In-111-labeled cells. The findings, combined with the histologic results, suggest that cellular rejection is the mechanism involved. They also suggest indirectly that neither Ga-67 citrate nor Tc-99m sulfur colloid would be an appropriate tracer for monitoring cardiac rejection. Previous reports in patients have shown Ga-67 citrate and Tc-99m sulfur colloid to be useful in detection of renal allograft rejection (*23*). The minimal increases in accumulation of these radionuclides during the late stages of cardiac allograft rejection (Figs. 2, 3) do not support a role for them in detecting such rejection.

Indium-111-labeled leukocytes and platelets are potentially useful for the detection of experimental cardiac allograft rejection by external imaging. In our hands, In-111-labeled leukocytes had a higher labeling efficiency than In-111-labeled platelets. These findings are similar to those of Scheffel et al (*21*). The fact that leukocytes are easier to isolate and label than platelets may favor their use in subsequent studies. However, the current data suggest that, with In-111 labeling, both platelets and leukocytes may be useful in evaluating cellular rejection in patients. The technique for monitoring allograft rejection used in this study is safe, simple, and noninvasive. The results suggest that further investigation of this approach is warranted in the evaluation of cardiac or other organ transplants.

FOOTNOTES

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