

## Intercellular Adhesion Molecule-1 Induction: A Sensitive and Quantitative Marker for Cardiac Allograft Rejection

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**Objectives.** Rats with abdominal heterotopic heart transplants were studied to determine whether cardiac allograft rejection could be assessed by immunoscintigraphy targeting intercellular adhesion molecule-1 (ICAM-1), which was induced on allografted organ cells in association with rejection.

**Background.** It is important to detect early rejection before development of myocyte necrosis. Although a variety of methods for the detection of cardiac rejection have been investigated, histologic inspection of biopsied samples is still used routinely for clinical diagnosis of rejection.

**Methods.** DA rat (*RT-1<sup>a</sup>*) hearts were transplanted into PVG rats (*RT-1<sup>c</sup>*). Immunohistologic examination of the allografts demonstrated that ICAM-1 induction on vascular endothelial cells was observed as early as 4 days after transplantation in this combination. Thirty-nine allografted rats and seven isografted rats were studied. One day after injection of 100  $\mu$ Ci of <sup>111</sup>In-

labeled anti-ICAM-1 monoclonal antibody (1A29), planar images were obtained.

**Results.** Rejecting allografts showed increased radiotracer uptake and could be identified on the images as early as 5 days after transplantation. In contrast, nonrejecting cardiac allografts and isografts did not show specific uptake. Mildly rejecting allografts, with mononuclear cell infiltration but without significant myocyte necrosis, could be scintigraphically identified, and the level of radiotracer uptake reflected the histologic severity of rejection. Accumulation of <sup>111</sup>In-labeled monoclonal antibody of isotype-matched irrelevant specificity was not detected in the rejecting allografts.

**Conclusions.** These data indicate that ICAM-1 induction can be assessed quantitatively by radioimmunoscintigraphy. Radioimmunoscintigraphy is a sensitive method for early detection and assessment of cardiac allograft rejection.

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Heart transplantation is an accepted treatment for patients with life-threatening heart failure. Rejection of the transplanted heart is a serious complication of this treatment. Detection of rejection requires histologic inspection of myocardial specimens obtained through endomyocardial biopsy (1). However, endomyocardial biopsy is invasive, costly, prone to sampling error and very dependent on the experience of the reader. To avoid these problems, noninvasive methods of detecting heart transplant rejection are desirable. Noninvasive detection of early rejection before development of myocyte necrosis should be advantageous for monitoring of patients.

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Scintigraphy targeting the immune reaction may provide an approach to solve this problem. This approach also may allow the assessment of pathophysiologic mechanisms of the rejection process.

We previously demonstrated (2-4) that rejecting cardiac and renal allografts can be detected by radioimmunoscintigraphy targeting donor-specific and nonspecific MHC class II antigens in murine models. Although rejected organs are visualized by this technique, clinical utility of this approach has not been evaluated.

Primary allograft transplant rejection of vascularized organs is mediated by T cells that attack endothelial and parenchymal cells bearing foreign histocompatibility antigens (5). Adhesion of T cells to vascular endothelium is a necessary step for activation of T cells. Intercellular adhesion molecule-1 (ICAM-1) is one of the adhesion molecules that activate T cells and mediate the adherence of T cells to antigen-presenting cells (6-9). ICAM-1 normally is expressed on vascular endothelial cells, fibroblasts, epithelial cells, macrophages and activated lymphocytes (10,11). It also is induced on allografted organ cells early in rejection (12-14). We previously showed (15) that ICAM-1 plays a crucial pathophysiologic role in rejection by working with lymphocyte function associated antigen-1 (LFA-1) (15). Therefore, the detection of

ICAM-1 induction may provide an early marker of immune activation in cardiac allograft transplantation. The present study was performed to determine whether radioimmune scintigraphy targeting ICAM-1 can be used to detect rejection of cardiac allografts. ICAM-1 induction was assessed immunohistologically and compared with induction of MHC class I and class II antigens.

## Methods

**Animals.** Inbred male rats (weight 150 to 200 g) of the PVG (*RT-1<sup>c</sup>*) strain were used as recipients, and those of the DA (*RT-1<sup>a</sup>*) strain were used as donors. They were purchased from a commercial source (Bantin & Kingman). All animal experiments were carried out according to National Institutes of Health guidelines.

**Monoclonal antibodies.** Hybridoma cells 1A29 (anti-rat ICAM-1, mouse IgG1) (16) were injected into nude mice to produce ascites. The antibody was purified by a protein G affinity column from ascitic fluids. Monoclonal antibody of irrelevant specificity (MOPC21, mouse IgG1; Sigma Corp.) was used as an isotype-matched control. WT.1 (anti-rat LFA-1, mouse IgG2a) (17) and F17-23-2 (anti-rat major histocompatibility complex [MHC] class II polymorphic determinant, mouse IgG1) monoclonal antibodies also were purified from ascitic fluids. The MN4-91-6 (anti-rat MHC class I polymorphic determinant, mouse IgG1) monoclonal antibody was purchased from Serotec. The F17-23-2 and MN4-91-6 monoclonal antibodies react with DA but not with PVG rats (18).

**Heart transplantation.** Heterotopic rat heart transplantation was performed according to a modification (4) of the method of Ono and Lindsey (19). Recipient and donor rats were anesthetized intraperitoneally with 50 mg/kg body weight pentobarbital sodium. Heterotopic heart transplantation was performed by anastomosing the donor aorta and pulmonary artery to the recipient abdominal aorta and inferior vena cava, respectively. The strength of the graft heart beat was checked daily by palpation.

**Animal groups.** Fifty-three DA hearts were transplanted into PVG recipients. Twenty-nine of them were randomly assigned for scintigraphy using <sup>111</sup>In-labeled 1A29 at 2 to 14 days after transplantation. Ten of the 53 allografted rats were treated with FK506 (1 mg/kg per day for 7 days, intramuscular injection). Five allografted rats without treatment were injected with <sup>111</sup>In-labeled MOPC21 as the control group. Nine allografted rats were used for immunohistologic study. Control scintigraphy also was performed by using <sup>111</sup>In-labeled 1A29 in seven isografted rats (DA to DA or PVG to PVG).

**Radioisotope labeling and scintigraphy.** Labeling and scintigraphy were performed as described (2,4,20). Briefly, purified 1A29 or MOPC21 was coupled to a bifunctional chelating agent, diethylenetriamine pentaacetic acid (DTPA), and the conjugate was labeled with indium-111 chloride immediately before injection (21). One hundred microcuries of radiotracer was injected into the tail vein 24 h before scintigraphy. Planar images were obtained digitally on a dedicated computer system

by using a gamma camera (Ohio-Nuclear Sigma 410) equipped with a parallel-hole medium energy collimator. The images were evaluated by two independent observers in a blinded manner.

**Biodistribution and histologic study.** Rats were killed after scintigraphy. Tissue biodistribution of radioactivity was determined as previously described (2,4,20). Graft, native heart, lung, liver, spleen, kidney and blood were collected and weighed. Radioactivity was counted by a gamma-well scintillation counter. The grafts were fixed in 10% neutral buffered formalin for hematoxylin and eosin staining. The samples identified by code were graded according to the working formulation of the International Society for Heart Transplantation (1) by a cardiac pathologist in a blinded manner.

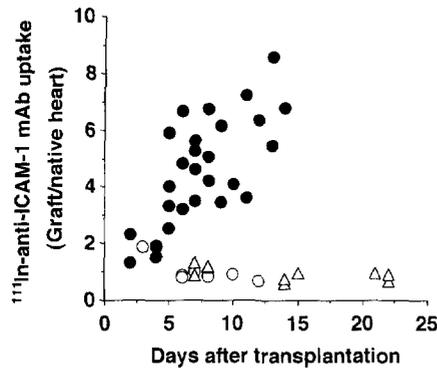
**Immunohistologic study.** Allografted hearts at 2 to 14 days after operation and normal DA rat hearts were studied immunohistologically. Samples were embedded in OCT compound (Sigma Corp.) and kept frozen in liquid nitrogen. Cryostat sections of 4  $\mu$ m were prepared, air-dried on poly-L-lysine-coated slides and fixed in acetone for 8 min. After blocking with normal rabbit serum (1:100 dilution in phosphate-buffered saline) at room temperature for 20 min, samples were incubated with mouse anti-rat IgG monoclonal antibodies (1A29, WT.1, F17-23-2 and MN4-91-6) at room temperature for 60 min, washed in phosphate-buffered saline and incubated with fluorescein isothiocyanate-conjugated rat serum-adsorbed rabbit anti-mouse IgG monoclonal antibody at room temperature for 30 min. After washing in phosphate-buffered saline, they were photographed under a Nikon fluorescence microscope.

Control samples were reacted without mouse anti-rat IgG monoclonal antibody incubation. Several samples were obtained from rats injected with <sup>111</sup>In-labeled 1A29 for scintigraphy. These samples were reacted in the same manner, skipping the incubation with a primary monoclonal antibody (1A29).

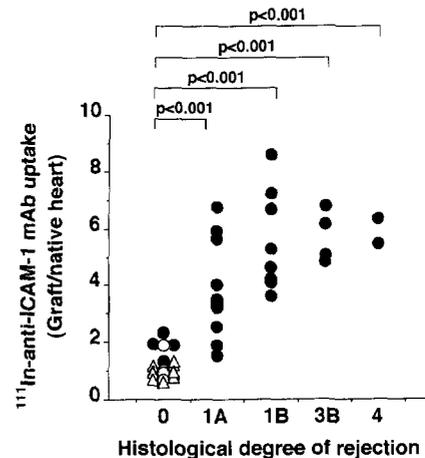
**Statistical analysis.** All data in the text and tables are expressed as mean value  $\pm$  SEM. Comparisons of data between the two groups of <sup>111</sup>In-labeled antibody were performed by the Mann-Whitney *U* test. Differences between the values of myocardial radiotracer uptake within each graft subgroup and the ratios within the five histologic degrees of rejection were compared by one-way analysis of variance of the Kruskal-Wallis test and the Scheffe *F* test; *p* < 0.05 was considered statistically significant. Statistical testing was performed using commercial software (StatView Version 4.02).

## Results

**Function and histologic examination of cardiac allografts.** The intensity of the cardiac impulse declined gradually in parallel with the progression of graft rejection. Nontreated cardiac allografts stopped beating between 15 and 20 days after transplantation. In contrast, all cardiac isografts and FK506-treated allografts continued beating at the time of sacrifice. Histopathologic examination of the allografts ranged from normal to severe rejection. Focal lymphocyte infiltration was



**Figure 1.** Time course of  $^{111}\text{In}$ -labeled 1A29 uptake in the grafts. The ratio of myocardial radiotracer uptake in the graft to that in the native heart increases progressively with time in the allografted rats without FK506 treatment (solid circles). In contrast, isografted rats (open circles) and allografted rats with FK506 treatment (triangles) did not show a significant increase in ratio throughout the course of observation. ICAM-1 = intercellular adhesion molecule-1; mAb = monoclonal antibody.



**Figure 2.** Comparisons of graft/native heart  $^{111}\text{In}$ -labeled 1A29 uptake versus histologic severity of rejection. Radiotracer uptake in allografts with grades 1A, 1B, 3B and 4 rejection is significantly greater than that in rats with nonrejecting grafts. Symbols and abbreviations as in Figure 1.

observed from 4 days after transplantation. Myocyte necrosis developed at ~6 to 8 days and progressed with time. At 12 to 14 days, myocyte necrosis with edema, hemorrhage and vasculitis was obvious.

**Uptake of  $^{111}\text{In}$ -labeled 1A29.** Uptake of  $^{111}\text{In}$ -labeled anti-ICAM-1 monoclonal antibody (1A29) in the allografted hearts without treatment ( $0.61 \pm 0.05\%$  injected dose/organ,  $n = 29$ ,  $p < 0.001$ ) was greater than that in the isografted rats ( $0.17 \pm 0.02\%$  injected dose/organ,  $n = 7$ ) or that in the allografted rats treated with FK506 ( $0.14 \pm 0.01\%$  injected dose/organ,  $n = 10$ ).

The ratio of myocardial  $^{111}\text{In}$ -labeled 1A29 uptake in the graft versus that in the native heart increased from day 5 of transplantation and accelerated with time after transplantation in the nontreated allografts (Fig. 1). The ratio of myocardial  $^{111}\text{In}$ -labeled 1A29 uptake in the graft versus that in the native heart in the nontreated allografts ( $n = 29$ ) ranged from 1.31 to 8.58. In contrast, the isografted rats ( $n = 7$ ) did not show a significant increase in ratio (0.70 to 1.90) as late as 12 days after transplantation. The allografted rats treated with FK506 ( $n = 10$ ) also did not show a significant increase in ratio (0.61 to 1.31) as late as 22 days after transplantation.

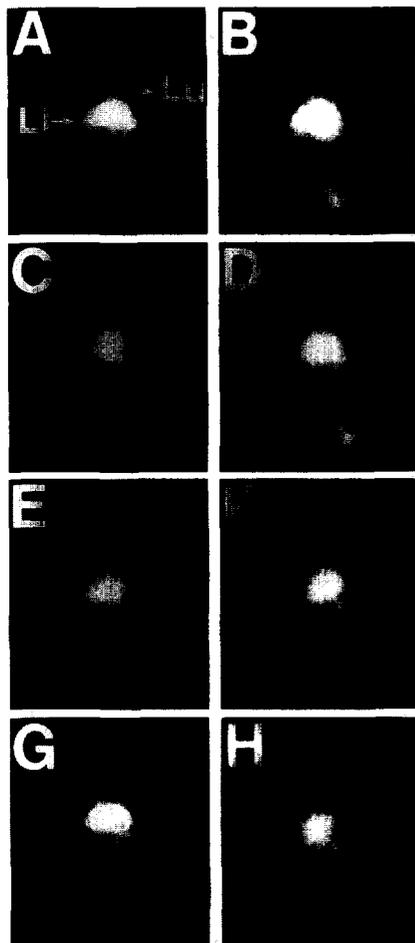
The level of the ratio reflected the histologic severity of rejection (Fig. 2). The ratio was  $1.14 \pm 0.10$  for normal grafts ( $n = 21$ ),  $3.79 \pm 0.50$  for grafts with grade 1A rejection ( $n = 11$ ,  $p < 0.001$  vs. normal),  $5.53 \pm 0.63$  for grafts with grade 1B rejection ( $n = 8$ ,  $p < 0.001$  vs. normal),  $5.70 \pm 0.46$  for grafts with grade 3B rejection ( $n = 4$ ,  $p < 0.001$  vs. normal) and  $5.91 \pm 0.44$  for grafts with grade 4 rejection ( $n = 2$ ,  $p < 0.001$  vs. normal).

**Uptake of  $^{111}\text{In}$ -labeled MOPC21.** Five allografted rats without treatment were imaged with  $^{111}\text{In}$ -labeled monoclonal antibody of irrelevant specificity (MOPC21) as a control group. Indium-111-labeled MOPC21 did not accumulate in mildly rejecting allografts ( $0.21 \pm 0.03\%$  injected dose/organ for 1A

[ $n = 3$ ] and 1B rejection [ $n = 2$ ] compared with  $^{111}\text{In}$ -labeled 1A29 uptake of rejected allografts with similar severity ( $0.60 \pm 0.07\%$  for 1A [ $n = 11$ ] and 1B rejection [ $n = 8$ ],  $p < 0.01$ ).

**Scintigraphic detection of cardiac rejection.** Visualization of the rejecting allografts on the scintigraphic images preceded the decline in graft beating. The rejecting allografts showed increased  $^{111}\text{In}$ -labeled 1A29 uptake and were scintigraphically identified as early as 5 days after transplantation (Fig. 3). Rejecting allografts with focal lymphocyte infiltration but without myocyte necrosis (grade 1A) could be clearly visualized (Fig. 3, B and C). Eight of the 11 allografts with grade 1A rejection could be identified. All 14 cardiac allografts with mild (grade 1B), moderate (grade 3B) and severe (grade 4) rejection showed intense and unequivocal accumulation of radiotracer (Fig. 3, D and E). In contrast, the nonrejecting cardiac allografts (Fig. 3A), including those in the FK506-treated rats (Fig. 3F) and isografted rats (Fig. 3G) did not show specific radiotracer uptake and could not be identified on the image. Rejecting allografts were not visualized by  $^{111}\text{In}$ -labeled MOPC21 (Fig. 3H).

Cardiac allograft rejection was detected by scintigraphy with 88% sensitivity (22 of 25 true positive results) and 100% specificity (21 of 21 true negative results). There were no false positive or false negative results for rejection grades 1B, 3B or 4. The threshold percent injected dose/organ value for a positive image was 0.40. This value was sufficiently greater than that for nonrejected allografts ( $0.32 \pm 0.02$ ) or isografts ( $0.17 \pm 0.02$ ). Percent injected dose/organ was  $>0.40$  in 7 of the 11 allografts with grade 1A rejection or all of the 14 allografts with grade 1B, 3B or 4 rejection. Percent injected dose/organ of two allografts with grade 1A rejection was  $<0.40$ . These grafts were not visualized. Percent injected dose/organ of the remaining two allografts with grade 1A rejection was 0.40: One graft was visualized, and the other was not.



**Figure 3.** Representative scintigrams after injection of  $^{111}\text{In}$ -labeled 1A29 (anti-ICAM-1 monoclonal antibody) in rats with a heterotopic heart transplant. A nonrejected allograft 4 days after transplantation (grade 0 rejection) is not imaged (A). Rejecting allografts at day 5 (B) (grade 1A), day 6 (C) (grade 1A), day 6 (D) (grade 3B) and day 8 (E) (grade 3B) are clearly visualized in the scintigrams (arrows). A nonrejected allograft with FK506 treatment (F) and an isograft (G) are not visualized.  $^{111}\text{In}$ -labeled MOPC21 (isotype-matched control monoclonal antibody with irrelevant specificity) does not accumulate in a rejecting allograft at day 8 (H) (grade 1A). Li = liver; Lu = lung.

**Biodistribution of  $^{111}\text{In}$ -labeled anti-ICAM-1 monoclonal antibody.** The results of radiotracer accumulation in the graft, recipient native heart, lung, liver, spleen, kidney and blood are shown in the Table 1. The  $^{111}\text{In}$ -labeled 1A29 uptake was significantly greater than  $^{111}\text{In}$ -labeled MOPC21 uptake in the lung, liver and spleen. No specific uptake of  $^{111}\text{In}$ -labeled 1A29 or  $^{111}\text{In}$ -labeled MOPC21 was found in the native heart.

**Expression of ICAM-1, LFA-1 and MHC antigens in cardiac grafts and normal hearts.** Results of semiquantitative analysis of ICAM-1 and MHC class I and class II antigen expression are presented in Table 2. ICAM-1 expression was observed on normal capillary endothelial cells but not on cardiac myocytes. Enhanced expression of ICAM-1 in cardiac allografts was observed on capillary endothelium from day 4 after transplantation before the advent of myocyte necrosis (Fig. 4A). This expression correlated with scintigraphic visual-

**Table 1.** Biodistribution of  $^{111}\text{In}$ -Labeled 1A29 and  $^{111}\text{In}$ -Labeled MOPC21 in Various Organs (mean  $\pm$  SEM)

FK506 Treatment	mAb 1A29 (% ID/g)		mAb MOPC21 (% ID/g): Nongrafted Rats, n = 7	
	Nongrafted Rats, n = 11	Allografted Rats n = 29* n = 10†		
Body weight (g)	227 $\pm$ 5	190 $\pm$ 5	202 $\pm$ 6	200 $\pm$ 21
Grafted heart	—	1.00 $\pm$ 0.06	0.34 $\pm$ 0.02	—
Native heart	0.25 $\pm$ 0.01	0.32 $\pm$ 0.01	0.33 $\pm$ 0.01	0.24 $\pm$ 0.30
Lung	5.71 $\pm$ 0.21	6.22 $\pm$ 0.25	5.97 $\pm$ 0.30	0.68 $\pm$ 0.07
Liver	5.18 $\pm$ 0.28	5.91 $\pm$ 0.16	5.35 $\pm$ 0.31	1.57 $\pm$ 0.21
Spleen	4.40 $\pm$ 0.22	5.47 $\pm$ 0.29	4.47 $\pm$ 0.27	2.15 $\pm$ 0.23
Kidney	2.77 $\pm$ 0.12	3.28 $\pm$ 0.09	3.28 $\pm$ 0.08	3.29 $\pm$ 0.43
Blood	0.33 $\pm$ 0.02	0.30 $\pm$ 0.01	0.33 $\pm$ 0.01	0.65 $\pm$ 0.13

\*Without, †with FK506 treatment. MAb = monoclonal antibody.

ization of rejecting allografts by  $^{111}\text{In}$ -labeled 1A29. Six to 8 days after transplantation, expression of ICAM-1 extended to the sarcolemma and was seen as late as 14 days (Fig. 4B). Allografts injected with 1A29 in vivo immediately before excision for scintigraphy showed the same pattern as those described previously. Infiltration of LFA-1 expressing cells was found from day 4 or 5.

Expression of MHC class II antigen in normal rat hearts and nonrejecting cardiac allografts was confined to interstitial dendritic cells. Induction of MHC class II antigen was observed on the capillary endothelium from day 4 after transplantation in parallel with the induction of ICAM-1 (Fig. 4C). The expression extended to the surface of myocytes and to the endothelium of small arteries on days 6 to 8 after transplantation (Fig. 4D). The expression of MHC class II antigen on myocytes declined with time. The distribution of MHC class I antigen in the normal rat heart was observed in the endothelium of small arteries and interstitial dendritic cells. Strong and diffuse expression was found on myocytes from days 6 to 8 and continued throughout the course of the study.

## Discussion

**Characteristics of ICAM-1 induction.** ICAM-1 mediates T cell adhesion to antigen-presenting cells and activates T cells working with its counterreceptor LFA-1 (7,8,22-24). It has been reported (12-14,25-29) that ICAM-1 is induced from the early stage of rejection in allografted liver, kidney and heart. Taylor et al. (26) demonstrated that an expression of adhesion molecules (ICAM-1, vascular cell adhesion molecule-1 [VCAM-1] and E-selectin), MHC class I and DR antigen on capillary endothelium increased during acute rejection. ICAM-1 also was induced on the myocardial membrane and intercalating disk. E-selectin and VCAM-1 represent early predictive markers of acute cardiac rejection, and ICAM-1 could be an important cofactor in both specific immune recognition and cellular recruitment (26,27). Tanio et al. (28) showed a strong correlation between ICAM-1 expression and the histologic severity of cardiac rejection in 25 heart trans-

**Table 2.** Expression of ICAM-1 and MHC class I and II Antigens in Cardiac Allografts

	Myocytes			Capillary Endothelium		
	MHC Class I	MHC Class II	ICAM-1	MHC Class I	MHC Class II	ICAM-1
Before transplantation	±	-	-	+	-	±
After transplantation						
2-3 d	±	-	-	+	±	±
4-5 d	+	±	±	+	+	+
6-8 d	+	+	+	+	+	+
9-14 d	+	±	+	+	+	+

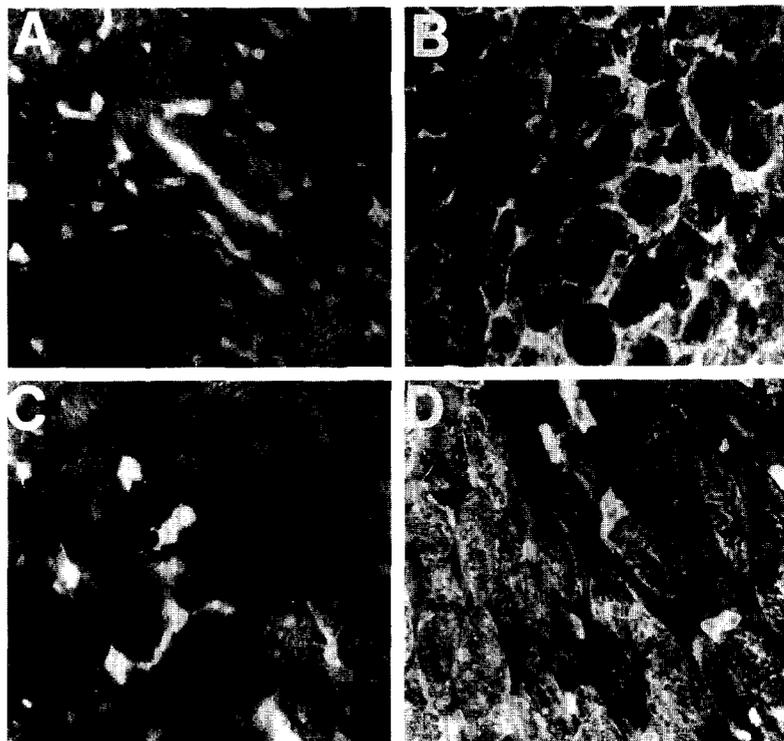
d = days; ICAM-1 = intercellular adhesion molecule-1; MHC = major histocompatibility complex; - = no expression; ± = faint expression on a few vessels or myocytes; + = strong expression on most vessels or myocytes.

plant recipients. We previously demonstrated (15,30) that short-term administration of monoclonal antibodies to ICAM-1 and LFA-1 allows induction of immunologic tolerance to cardiac allografts in mice. LFA-1 produces costimulatory signals that are required for optimal T-cell activation on binding with its ligand ICAM-1 (8). Therefore, induction of ICAM-1 should be one of the initial and essential events in the pathogenesis of rejection. The detection of ICAM-1 induction, therefore, could provide an early and specific marker of immune activation associated with acute rejection.

**Immunohistochemical comparison of ICAM-1 and MHC class II induction.** Immunohistologic studies from other laboratories (10,11,31) revealed that ICAM-1 is normally expressed on vascular endothelial cells, fibroblasts, epithelial cells, macrophages and activated lymphocytes. Although low levels of MHC class I antigen are expressed on normal cardiac myocytes, class II antigen is not detected (32). Induction of

donor-type class I and II antigens (18,33,34) and ICAM-1 (26-29) in rejecting cardiac allografts has been reported. Results of our immunohistologic studies generally confirmed these previous observations. Enhanced expression of ICAM-1 as well as MHC class II antigen on the capillary endothelium was observed from day 4 after transplantation in parallel with the development of mononuclear cell infiltration. The time course of induction of donor-type class II antigen and ICAM-1 was similar.

**Scintigraphic detection.** The level of radioactive anti-ICAM-1 monoclonal antibody uptake reflected the histopathologic severity of rejection. Rejecting allografts with no significant myocyte necrosis could be clearly identified by the scintigrams. There were no false positive images in the grafts without rejection. Because <sup>111</sup>In-labeled monoclonal antibody of irrelevant specificity did not accumulate in the rejecting allografts, it is unlikely that <sup>111</sup>In-labeled anti-ICAM-1 monoclonal antibody nonspecifically accumulated in sites of rejection.



**Figure 4.** Immunohistochemical study for ICAM-1 and MHC class II antigens in allografted hearts. An allograft 4 days after transplantation (rejection grades 1A [A and C]) and another allograft 10 days after transplantation (grade 3B [B and D]) are reacted with anti-ICAM-1 monoclonal antibody (A and B) and anti-MHC class II antigen monoclonal antibody (C and D). At day 4, focal expression of ICAM-1 (A) and MHC class II antigen (C) on the capillary endothelium as well as interstitial cells is demonstrated. An allograft with advanced rejection at day 10 shows strong expression of both ICAM-1 and MHC class II antigen on the sarcolemma of myocytes.

Grades 2 and 3A are the most common rejection types seen clinically. The rats in our study did not develop these two rejection grades, probably due to the difference between the two species in the progression of the rejection process, and to the modulation by immunosuppressive treatment.

Grade 1B rejection could not be distinguished from grades 3B or 4 rejection by radioimmunosintigraphy. Using this technique patients with 1B rejection might be overtreated, because 1B rejections are not usually treated clinically. Scintigraphy may be too sensitive in this respect. However, reliable noninvasive clinical tests for detecting early rejection should be beneficial. The frequency of endomyocardial biopsy could be reduced by radioimmunosintigraphy. In the present study, there were no false negative results for rejection grades 1B, 3B or 4. Also, we previously reported (20) that  $^{111}\text{In}$ -labeled antimyosin scintigraphy is a reliable technique for detecting grade 3B rejection. The combination of two noninvasive techniques by using two different isotopes might allow identification of early rejection with cellular infiltrates but without myocyte necrosis. Further experiments are required to test this possibility.

**Effects of FK506.** Rats treated with FK506 did not develop acute rejection, and  $^{111}\text{In}$ -labeled anti-ICAM-1 monoclonal antibody did not accumulate in the grafts. Expression of ICAM-1 in the allografts treated with FK506 could not be distinguished from that of normal heart. FK506 has been shown to inhibit production of T cell-derived lymphokines, such as interleukin-2 and interferon- $\gamma$  (35). FK506 is supposed to suppress induction of ICAM-1 and MHC class II antigen because induction of these molecules is certainly a consequence of local production of lymphokines released from infiltrating leukocytes (9,31,36). Therefore, it is reasonable that  $^{111}\text{In}$ -labeled anti-ICAM-1 monoclonal antibody uptake in FK506-treated allografts did not increase relative to that in isografts, reflecting the absence of active rejection.

**Reason for high background.** Higher accumulation of  $^{111}\text{In}$ -labeled anti-ICAM-1 monoclonal antibody was observed in the lungs and liver in normal rats as well as in those with rejecting cardiac allografts. Although the nature of this accumulation is a matter for further investigation, normal expression of ICAM-1 in pulmonary alveolar cells and vascular endothelium may account for it. Normal sinusoidal cells in liver have also been shown (37) to express significant amounts of ICAM-1. Indium-111-labeled monoclonal antibody of irrelevant specificity did not accumulate in the normal liver and lungs, suggesting that the increased lung and liver uptake were due to specific antigen recognition.

High background might cause difficulties when radioimmunosintigraphy is applied to orthotopic heart transplantation. Use of single-photon emission computed tomography would be helpful for improvement of spatial resolution. It is also possible to perform serial scintigraphy in individual patients. Comparisons of serial scintigraphic images would be useful for determining changes in radiotracer uptake in the cardiac graft. These possibilities should be further evaluated in orthotopic heart transplantation models.

**Comparison of radioimmune scintigraphy targeting MHC class II and ICAM-1 and other noninvasive methods.** We previously reported that radioimmune scintigraphy targeting MHC class II antigen allows detection of early rejection of cardiac (2,4) or renal allografts (3). In our previous investigation (4) we used  $^{123}\text{I}$ -labeled monoclonal antibodies against monomorphic or polymorphic determinants of MHC class II antigen in the same donor-recipient combination. Although the use of antipolymorphic portion monoclonal antibody offered better images, from a practical point of view the need for donor-specific monoclonal antibodies would limit the utility of the scintigraphy as applied to clinical cases. In contrast, anti-ICAM-1 monoclonal antibody can be applied to imaging of rejection irrespective of donor haplotype. Anti-MHC class II scintigraphy using the antimonomorphic portion monoclonal antibody was also performed using the same protocol as the present study. The ratio of myocardial radiotracer uptake in the graft to that in native heart for grade 1A rejection determined by this technique was 1.41 and 1.54, and that for grade 1B rejection 1.64 and 3.75. In the present study, however, the ratios for 1A and 1B rejection were  $5.53 \pm 0.63$  ( $n = 11$ , between 1.51 and 6.75) and  $5.70 \pm 0.46$  ( $n = 8$ , between 3.61 and 8.58), respectively. Anti-ICAM-1 imaging therefore appears to be more sensitive than anti-MHC class II imaging.

A variety of noninvasive methods for detection and quantification of heart transplant rejection have been investigated. Indium-111-labeled lymphocyte (38,39) has been used for imaging. However, correlation between severity of rejection and amount of radioactivity of this imaging agent has not been shown. Ferran et al. (27) suggested that few activated T cells expressing the interleukin-2 receptor are seen in intense infiltrates, but the expression is not predictive of rejection. Scintigraphy with  $^{111}\text{In}$ -labeled antimyosin is reported to be useful in detecting moderate and severe cardiac rejection (20,40,41). However, because myocyte necrosis associated with rejection is essential for imaging of antimyosin, this method is not sensitive enough to detect early heart transplant rejection without myocyte necrosis and not specific for immune activation.

**Implications.** The physiologic roles of ICAM-1 induction are still a matter of investigation. It has been reported that ICAM-1 is induced not only in rejecting organs, but also in tissues undergoing autoimmune injury (42) and inflammation (43) and in malignant tumors (44). ICAM-1 also has been shown to be a receptor of rhinovirus (45). The noninvasive detection of ICAM-1 induction presented in the present study could allow investigation of the pathophysiologic roles of the induction phenomenon in organ rejection and other immunologic disorders. Radioimmunosintigraphy is potentially applicable to the detection and localization of these disorders as well.

**Conclusions.** We demonstrated that the induced ICAM-1 resulting from rejection can be visualized *in vivo* by radioimmunosintigraphy, a noninvasive method can detect early rejection and allows its quantitative assessment. Radioimmunosintigraphy could be potentially useful for detection of cardiac allograft rejection in patients and would facilitate the

study of the mechanisms of interaction of ICAM-1/LFA-1 in various immunologic disorders. Further studies are needed to determine its clinical utility.

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