Cardiac Graft Intercellular Adhesion Molecule-1 (ICAM-1) and Interleukin-1 Expression Mediate Primary Isograft Failure and Induction of ICAM-1 in Organs Remote From the Site of Transplantation

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Abstract—During the first few hours after heart transplantation, the occurrence of graft failure is unpredictable and devastating. An explosive cascade of inflammatory events within the reperfused graft vasculature is likely to be mediated, at least in part, by the local expression of the leukocyte adhesion receptor intercellular adhesion molecule-1 (ICAM-1, CD54). Furthermore, although proinflammatory cytokines such as interleukin-1 (IL-1) are known to autoinduce their own (and ICAM-1) expression in vitro, there are no data to identify their functional in vivo cross talk in the setting of isograft transplantation. To determine the role of ICAM-1 in primary graft failure, we used an isogeneic vascularized model of heterotopic cardiac transplantation. ICAM-1 mRNA and protein increased in grafts during the early posttransplant period and were predominantly localized in the endothelium. The functional significance of this was established using donor hearts obtained from either ICAM-1–deficient (ICAM-1−/−) or control (ICAM-1+/+) mice. ICAM-1+/+ grafts exhibited increased neutrophil infiltration, reduced left ventricular compliance, and poorer survival than did ICAM-1−/− grafts. Increased ICAM-1 expression was not limited to ICAM-1−/− grafts but also occurred in unmanipulated recipient organs located remote from the site of surgery (but only after transplantation of ICAM-1−/−, not ICAM-1+/+, cardiac grafts). This expression of ICAM-1 in remote organs appeared to be triggered by IL-1α released from the graft, because (1) in situ hybridization revealed increased IL-1 mRNA within cells of the reperfused graft, including myocytes and endothelial cells; (2) ICAM-1 expression in remote organs coincided with a significant increase in serum levels of IL-1α after transplantation of ICAM-1+/+ grafts; both remote organ ICAM-1 expression and IL-1α levels were blunted by implantation of ICAM-1−/− grafts; and (3) remote organ ICAM-1 expression and neutrophil infiltration and IL-1 levels could be blocked by the administration of an IL-1 receptor antagonist. These data demonstrate an apparent positive-feedback loop in which local ICAM-1 and IL-1 expression leads to a mutual amplification of each other’s expression within the reperfused graft, promulgating inflammatory events that are likely to be an important cause of primary cardiac graft failure. Because IL-1 receptor blockade reduces the IL-1–mediated autoinduction of IL-1, reduces the expression of ICAM-1 in both the graft and remote organs, and improves graft survival, it may provide a new and effective strategy to prevent the occurrence of primary cardiac graft failure. (Circ Res. 1998;82:762-772.)

Key Words: cardiac transplantation ■ leukocyte adhesion receptor ■ interleukin-1 receptor antagonist ■ autoinduction
that is rapidly expressed at the endothelial surface from preformed storage pools, \(^6\) appears to mediate PMN-dependent tissue damage.\(^6\) However, at later time points, it is likely that other adhesion receptors whose expression may be induced in the proinflammatory vascular milieu of reperfusion may serve as the preponderant mechanisms responsible for PMN capture and graft demise. Of these other candidate leukocyte adhesion receptors, ICAM-1 is likely to be a key mediator of early graft failure, because its expression is inducible and because it has a high avidity for activated PMNs.\(^7\) Although ICAM-1–dependent PMN adhesion participates in the pathogenesis of cerebral injury in stroke\(^8\) and administration of blocking antibodies to ICAM-1, LFA-1, or Mac-1 in a recent study of heart transplantation in mice, the role of ICAM-1 expression in cardiac graft function was not established.\(^9\) Of these other candidate inducible and because it has a high avidity for activated PMNs.\(^7\) Although ICAM-1–dependent PMN adhesion participates in the pathogenesis of cerebral injury in stroke\(^8\) and because it has a high avidity for activated PMNs.\(^7\) Although ICAM-1–dependent PMN adhesion participates in the pathogenesis of cerebral injury in stroke\(^8\) and myocardial injury after infarction,\(^9\) its role in the setting of heart transplantation has not been established. In favor of a pathogenic role for ICAM-1 are studies in which the administration of blocking antibodies to ICAM-1, LFA-1, or Mac-1\(^1\)–\(^13\) resulted in improved cardiac graft function. However, in a recent study of heart transplantation in mice, the role of ICAM-1 expression in cardiac graft function was not established.\(^14\)

To account for the explosive inflammatory events that occur subsequent to reperfusion, there appear to be several inflammatory amplification mechanisms in the graft. IL-1, whose expression is augmented after a period of hypoxia,\(^15\) is a known potent inducer of endothelial ICAM-1 expression\(^16\) and is likely to be an important component of inflammatory upregulation in the reperfused graft. In addition, IL-1 is itself a potent autocrine for IL-1 gene expression in vascular smooth muscle cells.\(^17\)

The present study was designed to determine whether ICAM-1 expression increases in the early hours after cardiac transplantation, whether this is functionally relevant with respect to primary cardiac graft failure, and whether local ICAM-1 expression may amplify local and remote inflammatory events via a cytokine intermediary such as IL-1. Experiments were performed using a strategy wherein either control or deletionally mutant ICAM-1 murine donor hearts were transplanted into wild-type recipients that were syngeneic, so that the effects of preservation could be studied independent of potentially confounding effects of allograft-induced inflammation. In addition to identifying an important pathophysiological role of graft ICAM-1 expression in primary cardiac graft failure, the present experiments identify for the first time that ICAM-1 expression in the graft induces ICAM-1 expression at remote sites and that ICAM-1 may autoinduce its own expression by stimulating production of IL-1. Furthermore, these studies identify IL-1 receptor blockade as a novel means to reduce both local and remote ICAM-1 expression as well as to reduce the incidence of primary graft failure after transplantation.

**Materials and Methods**

**Mice**

ICAM-1–deficient mice, generated as previously reported,\(^18\) were backcrossed into C57Bl/6J mice to obtain homozygous null ICAM-1 mice. Mice from the fifth generation of backcrossings were used for this study (either ICAM-1\(^{-/-}\) or ICAM-1\(^{+/+}\) mice), conferring \(\sim96.9\%\) homogeneity for the C57Bl/6J background. For all of the experiments performed, recipient mice were ICAM-1\(^{+/+}\). Donor hearts were either ICAM-1\(^{-/-}\) or ICAM-1\(^{+/+}\), as indicated in the text. For all of the studies reported here, male mice that were 8 to 12 weeks of age and weighing 28 to 35 g were used.

**Heart Preservation and Transplantation**

Experiments were performed according to a protocol approved by the Institutional Animal Care and Use Committee at Columbia University. Transplantation was performed according to the Onolindsey model,\(^19\) as adapted for mice.\(^20\) Mice were anesthetized using intraperitoneal ketamine (50 mg/kg) and xylazine (10 mg/kg), after which a midline abdominal incision was made in the donor mouse, which was then heparinized through the inferior vena cava (50 U). The incision was extended cephalad to open the chest through a median sternotomy. The heart was rapidly harvested after arrest with potassium cardioplegia solution given via the inferior vena cava (1 mL, 20 mEq/L), and the coronary arteries were flushed (0.5 mL of preservation solution) and placed into lactated Ringer’s solution for 2 hours at 4°C. Transplantation was accomplished by exposing the recipient’s abdominal aorta and inferior vena cava by a similar long abdominal incision. The donor aorta and pulmonary artery were anastomosed, end to side, to the recipient’s abdominal aorta and inferior vena cava, respectively, using 10-0 nylon suture under \(\times 16\) magnification (Leitz Wild microscope M650, Wild Surgical Microscopes Co). During the transplantation procedure, the duration of warm ischemia was maintained constant (30 minutes), after which hearts were reperfused for the indicated durations.

**Assessment of Cardiac Graft Function and Survival**

At predetermined time points, grafts were considered to have survived if discrete rhythmic cardiac contractions could be discerned by palpation or, in the absence of a palpable impulse, if rhythmic electrical activity of the heterotopic heart could be established by ECG. In addition, using a scoring system developed for rat hearts,\(^21\) an observer blinded with respect to the experimental conditions assessed the cardiac grafts on the basis of contractility (0 to 2, \(2=\) best), color (0 to 2, \(2=\) pink), and turgor (0 to 1, \(1=\) soft) at the following times points after reperfusion: 10 minutes, 30 minutes (just before recipient abdominal closure), and again at 6 hours (just before graft explantation). Other than at these predetermined observation times, abdominal closure was maintained at all times so as to minimize temperature fluctuation and insensible fluid loss in the recipients. To reduce animal core temperature variability, core temperature was maintained at 37°C by using a rectal probe (sensor) interfaced with an infrared heating light.

To assess the effects of ICAM-1 expression on LV compliance, P-V relationships were determined immediately at the time of explantation by using a modification of a procedure previously described in rat hearts.\(^22\) A microvascular clamp was placed longitudinally on the abdominal vessels to isolate the donor heart from the recipient, and the transplanted heart was arrested at end diastole after the injection of 0.2 mL (20 mEq/L) of potassium chloride directly into the donor’s aortic root, proximal to the clamp, while the pulmonary artery was vented. The heart was rapidly excised and submerged in 4°C potassium (20 mEq/L) lactated...
Ringer’s solution, after which a 22-gauge angiocatheter connected to a three-way stopcock was inserted into the LV through the aortic valve, and the aortic root was occluded around the catheter at the level of coronary ostia with a 5-0 silk suture. Intracavitary LV pressures were measured with a 5F micromanometer (Millar Instruments, Inc) connected to the three-way stopcock. The third port of the stopcock was used for volume infusion, using a 2-mL microsyringe (Gilmet). After ensuring that all air was eliminated from the system, a small curved vascular clamp was placed right above the atrial side of the mitral annulus. The right ventricle was resected in order to determine LV compliance independent of the right ventricle. Volume was infused into the LV in 1-µL increments, with simultaneous recording of pressure using an analog-to-digital conversion and recording system (MacLab, MacLab Inc). Experiments were terminated when an LV pressure of 22.5 mm Hg was attained. To ensure that this method of measurement accurately measured compliance, volume was incrementally withdrawn to confirm that the P-V relationship did not change. All P-V relationship measurements were completed within 10 minutes of the onset of ischemia to avoid the onset of rigor.

mRNA Extraction and Northern Blot Analysis
In dedicated experiments, both native and donor hearts were rapidly excised and snap-frozen in liquid nitrogen until the time of mRNA extraction. After tissue homogenization using a Brinkmann Polytron homogenizer, total RNA was extracted from myocardial tissues using an RNA isolation kit (Stratagene). To detect ICAM-1 transcripts, equal amounts of RNA (20 µg/lane) were loaded onto an 0.8% agarose gel containing 2.2 mol/L formaldehyde for size fractionation and then transferred overnight by capillary pressure to nylon (Nitran) membranes with 10× SSC buffer. A murine ICAM-1 cDNA probe (1.90 kb, American Type Culture Collection) was labeled with [32P]dCTP by random primer labeling (Prime-A-Gene kit, Promega), hybridized to blots at 68°C, and washed twice with 2× SSC/0.05% SDS for 15 minutes and once with 0.1× SSC/0.1% SDS for 30 minutes. Blots were developed with X-Omat AR film exposed with a light screen at −80°C for 7 days.

Immunohistochemistry and In Situ Hybridization
Hearts were removed at the indicated times, fixed in 10% formalin, paraffin-embedded, and sectioned. Immunohistochemistry was performed by staining sections with a rat anti-murine ICAM-1 antibody (1:50 dilution) and then resuspended in EDTA-free buffer. Both sense and antisense probes were labeled with a horseradish peroxidase–conjugated secondary antibody detected with AEC (Sigma Chemical Co) as has been previously described. In situ hybridization was performed as follows: Murine IL-1α cDNA (pmIL1AcDNA) in pBluescript SK+ vector was purchased from the American Type Culture Collection. The RNA expression plasmid was linearized with BamHI and HindIII enzymes to allow in vitro run-off synthesis of both sense- and antisense-oriented RNA probes. The linearized plasmid was purified by phenol chloroform extraction and ethanol precipitation and then resuspended in EDTA-free buffer. Both sense and antisense probes were labeled by transcription with a digoxigenin RNA labeling Kit (Boehringer-Mannheim), and the labeled probes were then purified. Tissue sections were cut at 4 µm, floated on a water bath containing RNase-free water, and placed on glass slides precoated with opaque (VWR Scientific Products). Sections were dewaxed with xylene, rehydrated by immersion in graded concentrations of ethanol and diethyl pyrocarbonate–treated water, and immersed twice (5 minutes each time) in PBS and 100 mmol/L glycine at 37°C. Sections were delipidated with 0.3% Triton for 15 minutes and digested with 5 µg/mL proteinase K in Tris-EDETA (pH 8.0) for 30 minutes at room temperature. Sections were acetylated with 0.1 mol/L triethanolamine (pH 8.0) with 0.25% (vol/vol) acetic anhydride. Sections were then equilibrated for 60 minutes in hybridization buffer consisting of 4× SSC, 50% formamide, 5% dextran sulfate, 0.1 mg/mL yeast tRNA, and 0.05 mg/mL salmon sperm DNA. Hybridization was carried out overnight at 42°C with either a murine IL-1 sense or antisense probe (1.50 dilution in prehybridization buffer). Sections were subjected to stringent washes consisting of a single wash with 2× SSC, two 30-minute washes with 1× SSC at room temperature, two 30-minute washes with 0.1× SSC at 37°C, and two 20-minute washes Tris buffer (100 mmol/L Tris-HCl, 150 mmol/L NaCl). After blocking with blocking buffer (0.1% Triton X-100, 4% sheep serum, 100 mmol/L Tris-HCl, and 150 mmol/L NaCl), sections were incubated with a 1:500 dilution of anti-digoxigenin antibody (Boehringer-Mannheim) for 2 hours at room temperature. After four washes, color was allowed to develop for 2 hours, and development was stopped by dipping the slides briefly in Tris-EDETA buffer (pH 8.0) and then rinsing. Sections were counterstained lightly with 0.5% aqueous/dry mounting medium (BioMedo Corp) and covered with coverslips.

IL-1 Determination
Experiments in which IL-1α levels were measured were performed in three groups. In the first group, 1 mL of blood was drawn from the inferior vena cava of nonoperated (ICAM-1+/+) mice and centrifuged at 13 000 rpm for 10 minutes to obtain serum, which was then divided into aliquots and frozen at −80°C until the time of use. In the second group (sham-operated animals), an abdominal incision was performed, and the infrarenal abdominal aorta and inferior vena cava were cross-clamped for 35 minutes to simulate the ischemia that occurs during the heart transplantation procedure. At the termination of the ischemic period, the abdominal incision was closed, and 6 hours later, phlebotomy was performed and serum was prepared as described above. For these experiments, both ICAM-1+/+ and ICAM-1−/− mice were studied. In the third group, hearts were transplanted as described above, with serum samples obtained after 6 hours of observation. For these experiments, donor hearts from either ICAM-1+/+ or ICAM-1−/− mice were transplanted into ICAM-1+/+ recipients. IL-1α was assayed by ELISA (Endogen). The lower limit of detection of this assay is 6 pg/mL. Values are expressed as the mean±SEM of duplicate determinations.

IL-1ra Administration
For these experiments, donor hearts obtained from ICAM-1−/− mice were harvested and preserved identically as described above. Immediately before transplanting these hearts into ICAM-1+/+ mice, recipients were given recombinant human IL-1ra (20 µg/200 µL in PBS; endotoxin level, <0.001 ng/µg; R&D Systems) intravenously.

Figure 1. ICAM-1 mRNA levels after heart transplantation. mRNA was obtained either immediately after harvest of an unmanipulated heart from an ICAM-1+/+ mouse (fresh) or from hearts obtained from mice after transplantation. For these latter studies, the effects of reperfusion duration on ICAM-1 mRNA levels in transplanted (T) hearts and in the corresponding recipients’ native (N) hearts are shown. For all of these experiments, hearts were obtained from ICAM-1+/+ mice, preserved for 2 hours at 4°C, and transplanted into ICAM-1+/+ recipients (the duration of warm ischemia was kept constant at 30 minutes during the procedure, and the reperfusion duration is shown). Preparation of mRNA and Northern blotting were performed as described in “Materials and Methods.” Lanes were loaded with 20 µg RNA/lane, with equal loading confirmed by inspection of the 28S ribosomal RNA band by ethidium bromide staining. ICAM-1 mRNA is increased in the heterotopically transplanted heart, especially several hours after reperfusion, and is increased as well (albeit to a lesser degree) in the nonmanipulated native heart.
as an intraperitoneal injection. All surgical and postoperative procedures were identical between treated and nontreated groups.

Measurement of PMN Infiltration

MPO activity was measured as described. Briefly, tissue (hearts or kidneys) was homogenized with 2 mL HTAB, which releases the MPO enzyme from leukocyte granules. Freeze/thaw cycles were performed three times to further disrupt granules. Samples were centrifuged at 40 000g for 15 minutes at 4°C. Supernatant was collected and again centrifuged for 10 minutes with the same amount of HTAB, repeating the above steps one final time to obtain the final supernatant for assaying MPO activity. A 0.1 mL aliquot of this final clarified supernatant was added to 2.9 mL of substrate buffer with o-dianisidine hydrochloride (0.167 mg/mL, Sigma) and H2 O2 (30%, Sigma).

Data Analysis

MPO activity and transplantation scores were compared by using the Mann-Whitney U test for unpaired variables. Graft survival was evaluated by contingency analysis using the \( x^2 \) statistic. Statistical differences in the slope of the linear portion of P-V curves and serum IL-1\( \alpha \) levels were discriminated by ANOVA. All data are presented as mean\pm SEM. Statistical significance was defined as a value of \( P<.05 \).

Results

To determine whether there is an increase in ICAM-1 mRNA levels in cardiac grafts after transplantation, Northern blot analyses were performed using mRNA obtained from either fresh (nontransplanted) hearts or from ICAM-1 +/+ hearts that had been transplanted into ICAM-1 +/- recipients. Striking increases in ICAM-1 mRNA levels were seen in transplanted hearts (Figure 1, lanes marked “T”), peaking by 3 to 6 hours. To confirm that increased levels of mRNA were accompanied by increased expression of the ICAM-1 protein, immunohistochemistry was performed using a primary rat anti-murine ICAM-1 antibody on sections of cardiac tissue taken from ICAM-1 +/- grafts 6 hours after transplantation into ICAM-1 +/- recipients. ICAM-1 protein expression was demonstrated by the dark red endothelial staining pattern (Figure 2A). In contrast, when control ICAM-1 +/- grafts were transplanted into ICAM-1 +/- recipients and subjected to identical immunostaining procedures, no endothelial ICAM-1 staining could be identified in the transplanted hearts (Figure 2B). The absence of ICAM-1 protein is consistent with the fact that the ICAM-1 -/- mice have a targeted disruption of the ICAM-1 gene, which exists as a single-copy gene.

Although we initially wished to include mRNA obtained from the recipients’ native hearts as a control for Northern blotting, the data revealed that ICAM-1 levels in the native hearts increased over time after transplantation, despite the fact that these hearts had not been manipulated in any way (Figure 1, lanes marked “N”; these native hearts were obtained from the same animals from which the corresponding transplanted hearts were obtained). Additional experi-
icam-1 expression. 15,16 Serum obtained from ICAM-1 deficient cardiac grafts. demonstrated less remote ICAM-1 expression than did recipients of ICAM-1+/+ grafts (Figure 3, middle lane). Expression of ICAM-1 in these remote organs could not have been directly due to local tissue ischemia, because the aortic cross-clamping required for placement of the heterotopic cardiac graft was performed below the level of the renal arteries.

These data suggested the intriguing possibility that a circulating factor, perhaps a cytokine, was responsible for ICAM-1 expression in remote tissues. To test this possibility, serum levels of a candidate cytokine (IL-1α) were examined, because IL-1α is recognized to be a potent inducer of endothelial ICAM-1 expression.15,16 Serum obtained from control (nonoperated) mice or from those that had undergone a sham operation (35 minutes of abdominal aortic cross-clamping followed by 6 hours of reperfusion, to simulate the heart transplantation procedure) demonstrated that both ICAM-1+/+ and ICAM-1−/− mice had only slightly elevated IL-1α levels in the sham procedure (although IL-1 levels tended to be greater in the ICAM-1+/+ mice than in the ICAM-1−/− mice, P=.06) (Figure 4). However, after heart transplantation, there was a major increase in serum IL-1α, particularly in ICAM-1+/+ recipients that had received ICAM-1+/+ isografts (4.7-fold increase compared with nonoperated controls, P<.0005, Figure 4). Tumor necrosis factor-α, on the other hand, was not elevated under any of these experimental conditions (data not shown).

To determine whether the transplanted heart could be the source of IL-1 production, in situ hybridization experiments were performed using murine sense and antisense probes.22 In grafts subjected to 2 hours of cold preservation, 30 minutes of warm ischemia during transplantation, and 3 hours of reperfusion, endothelial cells exhibited the presence of IL-1 mRNA (Figure 5B and 5C, not seen in the sense controls [Figure 5A]). Review of multiple fields also revealed intense myocardial staining; this myocardial staining was not observed in any sense-stained heart (Figure 6A, 6C, and 6E) and was likewise not observed in nontransplanted antisense-stained control hearts (Figure 6B). When two time points were examined (Figure 6D and 6F), it appeared that the staining for IL-1 mRNA was most intense at 3 hours.

If IL-1 was indeed responsible for the expression of ICAM-1 in remote organs, we hypothesized that IL-1 receptor blockade might reduce the expression of ICAM-1 in remote organs. To test this hypothesis, experiments were performed in which recipient ICAM-1+/+ mice received an intravenous injection of IL-1ra immediately before the implantation of ICAM-1+/+ grafts. The preparation of IL-1ra that was used, recombinant human IL-1ra, has been previously shown to have functional blocking activity for IL-1 in mice29,30 and to be active against both IL-1 receptor subtypes.33,35 Mice receiving IL-1ra demonstrated reduced expression of ICAM-1 mRNA not only in native hearts but also within the transplanted heart itself, suggesting an additional autocrine role for IL-1 to promote local ICAM-1 expression within the graft (Figure 7). Not only did IL-1ra block the...
induction of ICAM-1 mRNA, but there was also an associated reduction of ICAM-1 protein expression by immunohistochemistry (Figure 8). The ability of IL-1ra pretreatment to reduce remote ICAM-1 expression in the recipient’s native heart was paralleled by its ability to reduce ICAM-1 expression in other remote organs as well, such as the recipient’s kidney (Figure 9A). To establish the functional relevance in remote organs, the cardiac graft recipients’ kidneys were also examined for neutrophil accumulation. Neutrophil accumulation was increased in kidneys of ICAM-1 +/+ recipients receiving ICAM-1 +/+ grafts (Figure 9B, solid bar) compared with either ICAM-1 +/- recipients receiving ICAM-1 +/- grafts (Figure 9B, open bar) or IL-1ra–pretreated ICAM-1 +/+ recipients receiving ICAM-1 +/+ grafts (Figure 9B, hatched bar). It is also interesting to note that serum obtained from IL-1ra–pretreated transplant recipients exhibited lower serum IL-1a levels than that from untreated animals (Figure 4, rightmost [hatched] bar), giving in vivo support for the in vitro observation that IL-1 may autoinduce IL-1 gene expression.17 Together, these data suggest that there exists a positive-feedback loop in which local ICAM-1 expression augments both local and remote ICAM-1 expression by an IL-1–dependent mechanism. This mechanism may serve to amplify the inflammatory response after heart transplantation.

To determine the functional significance of ICAM-1 expression in the cardiac isograft, grafts were evaluated for neutrophil infiltration, LV compliance, and survival 6 hours...
after the transplantation procedure. The effects of ICAM-1 expression on graft neutrophil infiltration were quantified by measuring the neutrophil-specific enzyme MPO. ICAM-1+/+ isografts showed significantly greater neutrophil accumulation than did ICAM-1−/− isografts at both the 3- and 6-hour time points after reperfusion (P<.05 and P<.005, respectively; Figure 10).

As an additional functional measure, LV diastolic compliance was determined in transplanted isografts that survived to the 6-hour time point after transplantation. In this procedure, intraventricular pressure measurements were obtained during incremental volume infusion, so that the slope of the P-V relationships could be obtained as a measure of LV compliance.24,25 Three groups of hearts were studied: (1) ICAM-1+/+ grafts transplanted into ICAM-1+/+ recipients, (2) ICAM-1−/− deficient grafts transplanted into ICAM-1+/+ recipients, and (3) nontransplanted control (ICAM-1+/+) hearts (excluding four grafts in which compliance could not be measured for technical reasons). Figure 11, left, shows representative tracings from grafts whose P-V slopes were the median for each group. P-V slopes from individual animals were then pooled for comparison of the data between each of the three groups of animals (Figure 11, right). Compliance of the ICAM-1+/+ grafts was significantly lower than that of ICAM-1−/− grafts, as indicated by the increased slope of the P-V relationship. In contrast, the compliance of the ICAM-1−/− grafts was similar to that of nontransplanted hearts.

To obtain a semiquantitative measure of graft function, a previously published scoring system was used21,22 in which transplantation scores were assigned at predetermined posttransplantation observation times by an observer blinded with

![Figure 7](http://circres.ahajournals.org/)

**Figure 7.** Effect of IL-1 receptor blockade on graft and remote ICAM-1 mRNA levels by Northern blot. For these experiments, the genotype of both donors and recipients was ICAM-1+/+. Grafts were preserved for 2 hours and reperfused for 6 hours before explantation. In one group of animals (designated (+)IL-1ra), recipients were pretreated with recombinant human IL-1ra (20 μg/animal, given as an intraperitoneal injection immediately before surgery). (-)IL-1ra indicates animals not treated with recombinant human IL-1ra; T, a transplanted heart; and N, the corresponding recipient’s native heart. These data demonstrate that treatment of the recipient with IL-1ra reduces both local (transplanted) and remote (native heart) ICAM-1 expression.

![Figure 8](http://circres.ahajournals.org/)

**Figure 8.** Effect of IL-1 receptor blockade on graft and remote ICAM-1 antigen expression detected by immunohistochemistry. For these experiments, the genotype of both donors and recipients was ICAM-1+/+. Grafts were preserved for 2 hours and reperfused for 6 hours before explantation. In one group of animals, recipients were left untreated (A), whereas in another group of animals (B), recipients were pretreated with IL-1ra recombinant human IL-1ra (20 μg/animal, given as an intraperitoneal injection immediately before surgery). Immunohistochemical identification of sites of ICAM-1 antigen expression revealed that IL-1ra reduces graft ICAM-1 antigen expression.
mice, only 48% of the isografts transplanted into ICAM-1/− mice (open bar) survived (P<.0001 between groups). In the ICAM-1 +/+ grafts, all of the graft failures occurred after 1 hour of reperfusion (Figure 13). However, if ICAM-1 +/+ hearts were transplanted into ICAM-1 +/+ recipients that were pretreated with IL-1ra (n = 8), graft survival was significantly improved (P<.05). In fact, survival of ICAM-1 +/+ grafts in IL-1ra–treated recipients was similar to that observed in the ICAM-1–deficient grafts (Figure 13).

Discussion

When a heart is transplanted into a human, it is critical that the graft function immediately and effectively in order to

As a final objective measure of the effects of ICAM-1 expression on cardiac graft function, graft survival was determined after transplantation. In these experiments, although there was 100% survival of ICAM-1 −/− isografts transplanted into ICAM-1 +/+ mice, only 48% of the ICAM-1 +/+ isografts transplanted into ICAM-1 +/+ mice

respect to the experimental conditions. At both 10 and 30 minutes after transplantation, transplantation scores of ICAM-1 −/− grafts were similar to those of ICAM-1 +/+ grafts (Figure 12). However, by 6 hours after reperfusion, the two groups had diverged, with ICAM-1−/− deficient grafts scoring considerably higher, indicating an improved functional outcome.

For these experiments, all recipient mice were ICAM-1 +/+ and, as experiments, all recipient mice were ICAM-1 +/+ , and the preservation duration was 2 hours. Donor genotypes, reperfusion durations, numbers of experiments, and P values are indicated in the figure. ∆Abs indicates change in absorbance.
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pretreated with IL-1ra.

incidence. The present experiments address both of these issues. ICAM-1 expression, which is increased in murine cardiac isografts after hypothermic preservation and transplantation, is functionally deleterious. Making matters worse, ICAM-1–bearing grafts synthesize and secrete IL-1, amplifying the proinflammatory response within the graft as well as triggering ICAM-1 expression at remote sites. In the present study, remote ICAM-1 expression (in the kidneys and perhaps other organs) is functionally relevant in that it is associated with increased leukosequestration at the remote site. A similar mechanism may occur soon after clinical heart transplantation, during which time patients often manifest unexplained pulmonary and renal dysfunction. Not only does local IL-1 production within the graft cause systemic effects, but it appears to be responsible for (1) autoinduction of IL-1, as has been reported in vascular smooth muscle cells in vitro,37 because IL-1 receptor blockade reduces plasma IL-1 levels, and (2) ICAM-1–mediated autoinduction of ICAM-1. As a final and critical issue, the present study identifies for the first time that a strategy of IL-1 receptor blockade is extremely effective at reducing local and remote ICAM-1 expression as well as enhancing survival of the transplanted heart. These data therefore identify IL-1ra administration as a novel treatment to reduce the incidence of primary graft failure.

There are several reasons why the present study focuses on ICAM-1, which is only one of several leukocyte adhesion receptors that may recruit PMNs to postischemic tissues. ICAM-1, a member of the immunoglobulin family of adhesion receptors, is a potent leukocyte adhesion receptor.2 It is constitutively expressed at low levels at the endothelial surface, but its expression may be augmented by a period of oxygen deprivation or ischemia, which greatly increases binding of neutrophils to endothelial cells.8,13,36 Although there are data to suggest that ICAM-1 contributes to the inflammatory response after myocardial infarction or stroke,8,50 in the setting of cardiac transplantation, the occurrence and functional significance of increased ICAM-1 expression are less well understood.10,14 By designing the present experiments so that inbred (syngeneic) mouse strains served as both donors and recipients of transplanted hearts, issues related to preservation/reperfusion could be isolated from immunological phenomena associated with allogeneic transplantation.

Although the present data support a role for increased ICAM-1 expression in the pathogenesis of cardiac dysfunction after transplantation, these data do not exclude an important complementary role of other adhesion receptors. P-selectin, which decreases circulating PMNs by promoting their rolling adhesion to endothelial cells,37 places ICAM-1 and LFA-1 in a favorable steric relationship to facilitate firm PMN adhesion. Preformed P-selectin may be rapidly expressed at the surface of hypoxic endothelial cells without the requirement for de novo protein synthesis,9 resulting in rapid PMN recruitment to postischemic tissues. Although not tested in the present study, it is possible that platelet-activating factor also facilitates the juxtaposition of LFA-1 and ICAM-1 after transplantation.39 Although the present data do not address the relative contributions of these other adhesion receptors, they do clearly identify the critical role of a single...
gene product, ICAM-1, in the pathogenesis of tissue injury after cardiac isograft transplantation. In this regard, it is interesting to note the time course in which most of the grafts failed in the present study—not within the first hour, but in the several hours thereafter. These data coincide with the time course of increased ICAM-1 expression after transplantation. In contrast, in previous experiments in which murine hearts were subjected to more profound ischemia before transplantation, primary graft failure was observed as early as 10 minutes after transplantation and appeared to be, at least in part, P-selectin dependent.7 These data support the concept that individual PMN adhesion mechanisms may have preponderant actions at different time points, corresponding to their differing modes and time courses of induction.

During the performance of the present study, an unexpected observation was made: ICAM-1 expression increases in remote organs after transplantation of ICAM-1–bearing (but not −deficient) grafts. Although this was first observed in the native hearts, follow-up experiments demonstrated that other organs, including the kidneys and the lungs, demonstrated similar increases in ICAM-1 expression after transplantation of ICAM-1–bearing grafts. These data suggest not only that ICAM-1 is expressed locally in the transplanted heart but also that there is a circulating factor(s) that induces ICAM-1 expression at distant sites. In pursuit of the underlying “factor,” we identified the proinflammatory cytokine IL-1α, a known stimulator of endothelial ICAM-1 expression,16 as the most likely candidate cytokine responsible for induction of ICAM-1 in remote organs. Not only did we identify increased graft IL-1 mRNA levels and increased serum IL-1α levels in the same conditions in which remote ICAM-1 expression was observed (after transplantation of ICAM-1 +/+ grafts), but we also noted that IL-1 receptor blockade effectively prevented remote ICAM-1 induction. For these experiments, we chose to use a recombinant form of a small naturally occurring peptide, IL-1ra.28,39,40 which is known to block the binding of IL-1 to either of its receptor subtypes.33–35 The form that we chose, recombinant human IL-1ra, has been previously shown to have functionally blocking properties in mice, where it is capable of reducing the severity of acute and chronic inflammatory conditions, including sepsis, colitis, and arthritis.29,30,39,41–47 The present study shows for the first time that IL-1ra is spectacularly effective at improving survival of the transplanted heart, which may be due to its effects to reduce ICAM-1 expression not only at remote sites but also within the graft itself. It is likely that in the context of organ transplantation, IL-1ra provides negative feedback to inhibit amplification of the local inflammatory response, inhibiting IL-1–mediated auto-induction of IL-1 gene expression as well as IL-1–mediated ICAM-1 induction in the graft and at remote sites. One cannot help being struck by the magnitude of apparent IL-1 production within the cardiac graft. If indeed IL-1 is a myocardial depressant factor, as many studies would appear to indicate,30–35 then one could also speculate that IL-1 receptor antagonism would directly improve cardiac performance in the early hours after heart transplantation. Furthermore, although it remains to be tested, these provocative data suggest that administration of IL-1ra may be therapeutically effective in other postischemic conditions as well, including myocardial infarction, stroke, and transplantation of other solid organs.

Taken together, these data are the first to clearly identify the pathogenic role of ICAM-1 in primary cardiac graft failure. IL-1 released from ICAM-1 +/+ grafts appears to amplify the local inflammatory response and to induce ICAM-1 expression at remote sites. Administration of a recombinant form of an endogenous peptide that is a competitive inhibitor of IL-1/IL-1 receptor interaction provides a novel pharmacological strategy to reduce the expression of ICAM-1 in remote organs as well as to reduce the occurrence of primary cardiac graft failure.

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Cardiac Graft Intercellular Adhesion Molecule-1 (ICAM-1) and Interleukin-1 Expression Mediate Primary Isograft Failure and Induction of ICAM-1 in Organs Remote From the Site of Transplantation

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