Upregulation of COX-2 During Cardiac Allograft Rejection

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Background—The hypothesis that cyclooxygenase-2 (COX-2) is involved in the myocardial inflammatory response during cardiac allograft rejection was investigated using a rat heterotopic abdominal cardiac transplantation model.

Methods and Results—COX-2 mRNA and protein in the myocardium of rejecting cardiac allografts were significantly elevated 3 to 5 days after transplantation compared with syngeneic controls (n=3, P<0.05). COX-2 upregulation paralleled in time and extent the upregulation of iNOS mRNA, protein, and enzyme activity in this model. COX-2 immunostaining was prominent in macrophages infiltrating the rejecting allografts and in damaged cardiac myocytes. Prostaglandin (PG) levels in rejecting allografts were also higher than in native hearts. Because NO has been reported to modulate PG synthesis by COX-2, additional transplants were performed using animals treated with a selective COX-2 inhibitor (SC-58125) and a selective inhibitor of the inducible nitric oxide synthase (iNOS) N-aminomethyl-L-lysine. At posttransplant day 5, inhibitor administration resulted in a significant reduction of COX-2 mRNA expression (3764±337 versus 5110±141 arbitrary units, n=3, P<0.05) and iNOS enzymatic activity (1.7±0.4 versus 22.8±14.4 nmol/mg protein, n=3, P<0.01) compared with vehicle-treated allogeneic transplants. Allograft survival in treated animals was increased modestly from 5.4 to 6.4 days (P<0.05). However, apoptosis of cardiac myocytes (TUNNEL method) was only marginally reduced relative to vehicle controls in treated graft recipients. The intensity of allograft rejection was also similar in the treated and untreated allografts.

Conclusions—The data indicates that COX-2 expression is enhanced in parallel with iNOS in the myocardium during cardiac allograft rejection. (Circulation. 2000;101:430-438.)

Key Words: prostaglandins ■ nitric oxide ■ rejection

Myocardial inflammation constitutes a major component of the pathologic changes observed during cardiac allograft rejection. Prostaglandins (PGs), along with leukotrienes and lipoxins, are lipid mediators which contribute to the vasodilation, edema, and plasma protein leakage which occur during the inflammatory response. Arachidonic acid released from cellular phospholipids by phospholipase A₂ is converted by the bis-oxygenase activity of cyclooxygenase to the prostaglandin endoperoxide PGG₂ and then to PGH₂. Prostaglandin H₂ is metabolized to biologically active products such as PGE₂, prostacyclin, and thromboxane A₂. It is known that cyclooxygenase exists in at least 2 isoforms. The constitutive isoform, COX-1, is believed to be responsible for the constitutive production of PGs such as PGI₂ by endothelial cells and the gastric mucosa and thromboxane A₂ by platelets. The other isoform, COX-2, is not constitutively expressed in most tissues but can be induced in endothelial cells, fibroblasts, smooth muscle cells, and macrophages by proinflammatory cytokines, endotoxin, PGs, tumor promoters, mitogens, and hypoxia. It is believed that COX-2 is induced during both acute and chronic inflammatory responses and is primarily responsible for the PG synthesis that ensues. This has led to the development of drugs that selectively inhibit PG production by COX-2, avoiding the adverse consequences, such as gastric ulcers, that may result from inhibition of COX-1.

In different inflammatory settings, the expression of COX-2 can be deleterious or protective. It has been reported that the activity of COX-1 and COX-2 can be enhanced by NO, augmenting the inflammatory response. Conversely, PGE₂ produced by the inducible isoform of COX can inhibit expression of inducible nitric oxide synthase (iNOS). The induction of COX-2 in endothelial cells increases the synthesis of PGI₂ thereby augmenting its protective actions to inhibit platelet aggregation, promote vasodilation, and reduce monocyte adhesion and activation on endothelial surfaces.

In previous studies we demonstrated that during cardiac allograft rejection in rats, iNOS mRNA, protein, and enzyme activity are induced. Using immunostaining, iNOS protein was demonstrated in infiltrating macrophages and lympho-
cytes, endothelial cells, vascular smooth muscle cells, and cardiac myocytes within the rejecting cardiac allografts. This work was confirmed by other groups who found that iNOS is expressed in macrophages and smooth muscle cells in the vasculopathic coronary arteries of allografts undergoing chronic rejection.\textsuperscript{10–12} iNOS mRNA and positive immunostaining have also been demonstrated in human cardiac allografts during rejection.\textsuperscript{13} NO produced by iNOS in this setting may impair contractile properties of the ventricle, reduce cardiomyocyte viability, and modulate the development of transplant vasculopathy.\textsuperscript{9–11,14,15} It may also modulate PG synthesis.\textsuperscript{3,6}

Accordingly, the present study was designed to investigate in a rat heterotopic heart transplantation model whether

**Methods**

**Cardiac Transplantation and Drug Treatment**

Male Lewis (TR-11) and Wistar-Furth (WF, RT-lu) rats weighing 180 to 200 g each were purchased from Harlan Sprague-Dawley Inc (Indianapolis, Ind). Syngeneic (Lewis-Lewis) and allogeneic (Lewis-Wistar-Furth) heterotopic abdominal cardiac transplantation was performed as previously described.\textsuperscript{9,16} Heart grafts in the abdomen were palpated daily. Rejection was determined by the lack of a heart beat and confirmed by inspection at laparotomy and by histological examination. Drug administration was begun on day 1 posttransplantation and continued until each assessment day (days 1, 3, and 5). SC-58125, [1-[(4-methylsulfonyl) phenyl]-3-trifluoromethyl-5-[(4-fluorophenyl)pyrazole] (G.D. Searle/Montsanto Co., St. Louis, Mo), which selectively inhibits mouse COX-2 enzyme (IC\textsubscript{50} 0.07 μM) without inhibiting COX-1 (IC\textsubscript{50} >100 μM) was given at a dosage of 10 mg · kg\textsuperscript{-1} · wk\textsuperscript{-1} (1 dose per week, i.p.) in a suspension in 0.5% aqueous methylcellulose and 0.025% Tween-20. The selective iNOS inhibitor, N-aminomethyl-L-lysine (L-NIL) (G.D. Searle/Montsanto Co, St. Louis, Mo), was given at a dose of 10 mg · kg\textsuperscript{-1} · d\textsuperscript{-1} (BID, i.p.). Animals were randomly grouped into drug and vehicle (0.9% saline) treatments.

**Histology and Labeling of Apoptotic Cells**

Hearts were fixed in 10% phosphate buffered formalin, embedded in paraffin, and 4 μm thick sections were cut and mounted on sialine-coated slides. For routine histologic examination, sections were stained with hematoxylin and eosin to determine the extent and severity of rejection according to the International Society of Heart and Lung Transplantation classification (ISHLT).\textsuperscript{17} Apoptotic cells were detected by in situ end-labeling, which detects the abundant DNA fragments in apoptotic nuclei using biotinylated deoxyuridine 5-triphosphate as described previously.\textsuperscript{18,19} Sections were also labeled for muscle actin with monoclonal antibody HHF-35 (Dako, Carpinteria, CA) using an immunoperoxidase technique which stained the cytoplasm of cardiac myocytes brown. The same immunoperoxidase technique was used to characterize the inflammatory infiltrate by labeling for T cell markers (anti-CD3) and macrophages (ED1).
Figure 4. Native rat heart (A and C) and rat heterotopic abdominal allograft (B and D) 5 days posttransplantation labeled for COX-1 (A and B) and COX-2 (C and D) represented by brown reaction product. The native hearts show COX-1 positive endothelial and endocardial cells (A). Cox-2 staining is weak in native hearts, where it is present in rare capillaries (C). Myocytes of native hearts are consistently COX-1– and COX-2–negative. During cardiac allograft rejection, COX-1 is also expressed in damaged cardiac myocytes in addition to endothelial cells (B) but not in infiltrating inflammatory cells (arrow). COX-2 is also upregulated in damaged cardiac myocytes (circle) and vascular smooth muscle cells (not shown). Magnification ×400.
COX-2 Immunohistochemistry

The RR6 monoclonal anti-mouse-COX-2 antibody was obtained from Dr Peter Isakson (G.D. Searle/Montsanto Co., St. Louis, Mo).20 The monoclonal anti-rat COX-1 antibody was obtained from Accu-rate Chemical Co (Westbury, NY). Immunohistochemistry was performed as previously described.20 Sections were then incubated with the anti-COX-2 Ab (clone RR6) diluted 1:150 in 5% horse serum PBS overnight at 4°C. Binding of the primary Ab to COX-2 was detected with the avidin-biotin-peroxidase technique labeling the site of the target antigen (COX-2) brown. For the COX-1, the primary antibody was diluted with 3% horse serum in PBS up to 1:150 and incubated at 4°C overnight. Horse anti-mouse secondary antibody was diluted 1:200 (Vector Laboratories, Burlingame, CA) and incubated for 30 minutes. Normal mouse serum was used as a negative control.

COX-2 and iNOS mRNA Ribonuclease Protection Assay

Specific mRNAs for COX-2 and iNOS were quantified by ribonuclease protection assay (RPA). Assay reagents and the procedures used were from an Ambion RPAII™ kit (Ambion Inc, Austin, Tex). The plasmid DNA used as a template for the rat iNOS probe was generously provided by Charles Rodi, Searle/Montsanto, St. Louis, Mo.

For the COX-2 RPAs, frozen graft or native hearts were thawed in guanidine isothiocyanate. Total RNA was isolated using the Ambion To-Tally™ RNA kit. Samples of total RNA were hybridized, digested, and separated. After electrophoresis, the gel was fixed and dried. Band intensities were quantified by electronic autoradiography using a Packard Instant Imager. The plasmid DNA used as a template for the rat COX-2 probe was graciously provided by P. Worley, Johns Hopkins School of Medicine, Baltimore, Md.

COX-2 Enzyme Protein Assay

The excised hearts were rinsed and flushed via the aorta with ice-cold saline to completely remove blood, then immediately frozen at −70°C. The frozen ventricular tissue was homogenized at 4°C in RIPA-lysis buffer supplemented with 10 μg/mL antipain, leupeptin and trypsin-inhibitor, and 0.1 mg/mL phenylmethylsulfonyl fluoride. Homogenates were centrifuged at 16,000 g for 30 minutes at 4°C. The protein concentration of supernatants were determined by BCA protein assay (Pierce) with BSA as standard. The total protein equivalents (40 μg per lane) for each sample were separated by 8% SDS-PAGE and electrotransferred to nitrocellulose membrane. After blocking nonspecific binding with TBS buffer containing 8% nonfat dried milk and 2% BSA, the membranes were immunoblotted with a mouse COX-2–specific (clone RR6) monoclonal antibody at a dilution of 1:2000 (1000-fold selectivity for mouse COX-2 compared with mouse COX-121). The blots were subsequently incubated with a horseradish peroxidase-conjugated secondary antibody and detected by the enhanced chemiluminescence method (DuPont NEM). The level of COX-2 protein was quantified using densitometric analysis (NIH image 1.60 software).

iNOS Enzyme Protein and Activity Assay

iNOS enzyme protein was measured as described above for the COX-2 protein assay. The anti-mouse iNOS polyclonal antiserum was a gift from Dr Mark Currie, G.D. Searle/Montsanto Co. iNOS enzyme activity was measured as previously described.9,18

Prostaglandin Assay

Both transplanted and native hearts were removed, cut into 2 slices, and immediately immersed in a HEPES-buffered Krebs solution. One slice was incubated in oxygenated Krebs solution only, the other was incubated in oxygenated Krebs solution containing bradykinin (100 μM) at 37°C, for 30 minutes. The supernatant was collected, mixed with water and methanol (10%), centrifuged at 4°C for 15
minutes at 375 g and loaded on a C18 cartridge (Millipore, Bedford, MA). This was followed by serial washings with deionized water, 10% methanol, petroleum ethyl, and elution with redistilled ethyl acetate, followed by evaporation of the organic phase under nitrogen. After suspending the pellets in phosphate buffer, prostaglandin E2 in the samples was measured with the TiterZyme PGE2 kit (PerSeptive Biosystems, Framingham, Mass).

Statistical Analysis
The time course of changes in COX-2 mRNA and protein and iNOS mRNA levels and enzyme activity were analyzed by ANOVA. PGE2 values were analyzed by ANOVA after log transformation due to the markedly nonnormal distribution of the values. The degree of apoptosis at each of the time points was analyzed using the nonparametric Kruskal-Wallis procedure. Survival rates were compared using the Mann-Whitney test.

Results
The expression of COX-2 mRNA was significantly increased in the rejecting cardiac allografts in comparison to syngeneic grafts on days 3 to 5 following transplantation (Figure 1). The COX-2 protein was also significantly upregulated in the rejecting allografts on days 4 to 5 posttransplantation (Figure 2). COX-1 mRNA was also increased in rejecting allografts on days 3 to 5 (Figure 3). To assess PG production in the rejecting graft tissue, myocardial slices from day 5 cardiac allografts and from the native hearts of the same rats were incubated in oxygenated buffer alone and in oxygenated buffer containing bradykinin (100 μmol/L). The mean PGE2 concentration per milligrams protein in the incubation media was significantly higher (P<0.05) in rejecting allografts in comparison to nonrejecting native hearts under control conditions, 474±678 versus 135±156 pg/mg of tissue and after bradykinin treatment, 1434±2451 versus 321±309 pg/mg of tissue (n=10).

Positive immunostaining for COX-2 was not observed in native hearts and syngeneic hearts other than a slight staining of rare endothelial cells (Figure 4C). COX-2 immunostaining in rejecting allografts was markedly increased in macrophages, damaged cardiomyocytes and in endothelial cells and smooth muscle cells especially in myocardial regions with an inflammatory infiltrate (Figure 4D). The increased expression of COX-2 mRNA (Figure 1) was similar in time and extent to the expression of iNOS mRNA in the allografts (Figure 5).

Positive immunostaining for COX-1 was apparent in endothelial and endocardial cells of the native hearts (Figure 4A). In rejecting allografts, COX-1 immunostaining was increased in endothelial cells. It was not observed in macrophages but was present in damaged cardiomyocytes (Figure 4B).

Treatment of the allograft recipients with L-NIL had no significant effect on COX-2 protein levels in the rejecting allografts but was associated with a reduction in iNOS enzyme activity (Figures 6B and 7). Treatment with the combination of SC-58125 and L-NIL was associated with a reduction of COX-2 protein in the 5-day rejecting allografts of 10% to 15% (Figure 6B). Survival of the cardiac allografts treated with the 2 inhibitors was increased slightly but significantly from 5.4±0.5 to 6.4±0.5 days (n=8, P<0.05). However, both the allografts treated with vehicle and the allografts treated with the inhibitors (Figure 8) showed severe inflammation and multiple foci of myocyte damage at day 5.

Figure 8. Cardiac allografts 5 days posttransplantation treated with vehicle (a) or with SC-58125 and L-NIL (b). Both allografts show severe inflammation and multiple foci of myocyte damage. Magnification ×250.
In the SC-58125 plus L-NIL–treated animals, COX-2 immuno-
staining was most apparent in the infiltrating macrophages,
with decreased immunostaining of cardiac myocytes (Figure 9).
In both treated and untreated cardiac allografts, the number of
apoptotic cardiac myocytes and the total number of
apoptotic nuclei increased exponentially during rejection
(Figure 10). There was no significant difference in the mean
numbers of apoptotic nuclei in treated (n = 6) versus untreated
(n = 3) allografts (16.1 ± 9.6 versus 7.5 ± 4.5) at day 5. Similarly,
there was no significant reduction in the rejection grade
of the treated cardiac allografts (Table).

Discussion
The data in this study indicate that during cardiac allograft
rejection COX-2 mRNA and protein are upregulated signif-
ically in the rejecting allografts at days 3 to 5 following
transplantation, in comparison to the levels observed in
syngeneic grafts at the same time points. Prostaglandin
synthesis was also significantly higher in myocardial tissue
from the rejecting allografts 5 days after transplantation in
comparison to that observed in native hearts. Immunostaining
indicated that COX-2 protein was markedly expressed in
endothelial cells, vascular smooth muscle cells, macrophages
infiltrating the myocardium, and focally in cardiac myocytes
that appeared damaged. The upregulation of COX-2 during
cardiac allograft rejection in this rat model paralleled in time
and extent the upregulation of iNOS mRNA, protein, and
enzyme activity.9,18 COX-1 protein was also significantly
increased at days 3 to 5, probably due to increased expression
of COX-1 in endothelial cells.

The expression of COX-2 together with that of iNOS has
previously been noted in a model of ureteral obstruction in
rats which leads to hydronephrosis and renal inflammation,22
in rat adjuvant arthritis,21 in carrageenin-induced pleurisy,23
in a model of subcutaneous inflammation produced by air
injection24,25 in human osteoarthritis-affected cartilage,26 and
in rat hearts following treatment with lipopolysaccharide.27
The co-induction of COX-2 and iNOS has also been observed
in studies in vitro of rat vascular smooth muscle cells,28,29
glomerular mesangial cells,7 murine macrophages,30 rat islets
of Langerhans,31 human endothelial cells,32 articular chond-
crocytes,33 and rabbit hepatocytes34 incubated with endotoxin
and/or cytokines but not in similar studies of human fetal cell
fibroblasts6 or bovine aortic endothelial cells.35 Proinflamma-
tory cytokines known to be synthesized and released by T
lymphocytes and macrophages during cardiac allograft rejec-
tion are probably responsible for induction of COX-2 in this
situation.26 In studies of porcine endothelial cells, exposed to
xenoreactive antibodies and complement, IL-1β mediated the
upregulation of COX-2 and synthesis of PGE2 and TXA2.37 In
studies of neonatal ventricular myocytes, IL-1β induced
iNOS, COX-2 mRNA, and protein along with a 200-fold
increase in PGE2.38

The relationship between the cyclooxygenase and NO
pathways varies depending on the circumstances. In cultured
bovine endothelial cells, NO or NO donor drugs have been
shown to inhibit PGI_{2} release by bradykinin (COX-1)\textsuperscript{39} and to inhibit COX-2 induction and activity in rat Kupffer cells.\textsuperscript{34} In contrast, however, NO and NO donor drugs have been shown to stimulate COX-1 and COX-2 activity in endotoxin-activated murine macrophages\textsuperscript{6} and in vascular smooth muscle cells\textsuperscript{28} and human endothelial cells.\textsuperscript{39,40} Similarly, in the hydronephrotic model of renal inflammation,\textsuperscript{22} in air-pouch–induced inflammation,\textsuperscript{24} there is evidence that NO augments the activity of COX-1 and COX-2, leading to enhanced synthesis of prostaglandins. The mechanism responsible for the effect of NO on COX activity is unclear but may involve nitrosylation of a cysteine residue in the active site of the COX enzymes,\textsuperscript{41} leading to the formation of nitrosothiols; these can produce structural changes in the enzyme, leading to increased COX catalytic efficiency.\textsuperscript{42} In other studies, it was demonstrated that NO enhanced the IL-1\textbeta-induced expression of the COX-2 mRNA and protein.\textsuperscript{43} The cytokine and endotoxin upregulation of COX-2 and iNOS in various cells and tissues is suppressed by dexamethasone and by other immunosuppressive drugs such as cyclosporin A and FK506.\textsuperscript{4,10,22,25,44} As mentioned previously, endothelial cell expression of COX-2 may be vasculoprotective by augmenting PGI_{2} synthesis.\textsuperscript{8} The finding of increased myocardial fibrosis in COX-2 knockout mice suggests that endocardial endothelial cell COX-2 may also be protective by augmenting PGI_{2} production.\textsuperscript{45}

In the present study, potent selective inhibitors of COX-2 and of iNOS were administered to rats undergoing cardiac transplantation.\textsuperscript{21,46} The administration of SC-58125 together with L-NIL resulted in the downregulation of the expression of COX-1 and COX-2, leading to enhanced synthesis of prostaglandins. The mechanism responsible for the effect of NO on COX activity is unclear but may involve nitrosylation of a cysteine residue in the active site of the COX enzymes,\textsuperscript{41} leading to the formation of nitrosothiols; these can produce structural changes in the enzyme, leading to increased COX catalytic efficiency.\textsuperscript{42} In other studies, it was demonstrated that NO enhanced the IL-1\textbeta-induced expression of the COX-2 mRNA and protein.\textsuperscript{43} The cytokine and endotoxin upregulation of COX-2 and iNOS in various cells and tissues is suppressed by dexamethasone and by other immunosuppressive drugs such as cyclosporin A and FK506.\textsuperscript{4,10,22,25,44} As mentioned previously, endothelial cell expression of COX-2 may be vasculoprotective by augmenting PGI_{2} synthesis.\textsuperscript{8} The finding of increased myocardial fibrosis in COX-2 knockout mice suggests that endocardial endothelial cell COX-2 may also be protective by augmenting PGI_{2} production.\textsuperscript{45}

In the present study, potent selective inhibitors of COX-2 and of iNOS were administered to rats undergoing cardiac transplantation.\textsuperscript{21,46} The administration of SC-58125 together with L-NIL resulted in the downregulation of the expression of COX-2 protein in the treated allografts, a finding that has been reported previously using an arthritis model in rats.\textsuperscript{21} The explanation for this is unclear but may reflect a role for PGs in the enhancement of COX-2 expression. The administration of L-NIL was associated with a marked reduction of iNOS enzyme activity in the rejecting allografts, a finding that has been reported previously using an arthritis model in rats.\textsuperscript{21} The explanation for this is unclear but may reflect a role for PGs in the enhancement of COX-2 expression. The administration of L-NIL was associated with a marked reduction of iNOS enzyme activity in the rejecting allografts, a finding that has been reported previously using an arthritis model in rats.\textsuperscript{21} The explanation for this is unclear but may reflect a role for PGs in the enhancement of COX-2 expression. The administration of L-NIL was associated with a marked reduction of iNOS enzyme activity in the rejecting allografts, a finding that has been reported previously using an arthritis model in rats.\textsuperscript{21} The explanation for this is unclear but may reflect a role for PGs in the enhancement of COX-2 expression. The administration of L-NIL was associated with a marked reduction of iNOS enzyme activity in the rejecting allografts, a finding that has been reported previously using an arthritis model in rats.\textsuperscript{21} The explanation for this is unclear but may reflect a role for PGs in the enhancement of COX-2 expression. The administration of L-NIL was associated with a marked reduction of iNOS enzyme activity in the rejecting allografts, a finding that has been reported previously using an arthritis model in rats.\textsuperscript{21} The explanation for this is unclear but may reflect a role for PGs in the enhancement of COX-2 expression. The administration of L-NIL was associated with a marked reduction of iNOS enzyme activity in the rejecting allografts, a finding that has been reported previously using an arthritis model in rats.\textsuperscript{21} The explanation for this is unclear but may reflect a role for PGs in the enhancement of COX-2 expression. The administration of L-NIL was associated with a marked reduction of iNOS enzyme activity in the rejecting allografts, a finding that has been reported previously using an arthritis model in rats.\textsuperscript{21} The explanation for this is unclear but may reflect a role for PGs in the enhancement of COX-2 expression. The administration of L-NIL was associated with a marked reduction of iNOS enzyme activity in the rejecting allografts, a finding that has been reported previously using an arthritis model in rats.\textsuperscript{21} The explanation for this is unclear but may reflect a role for PGs in the enhancement of COX-2 expression. The administration of L-NIL was associated with a marked reduction of iNOS enzyme activity in the rejecting allografts, a finding that has been reported previously using an arthritis model in rats.\textsuperscript{21} The explanation for this is unclear but may reflect a role for PGs in the enhancement of COX-2 expression.

| Grades of Rejection in Control and Treated Rat Cardiac Allografts |
|---------------------------------|-----------------|-----------------|
| Control | Treated With L-NIL and SC-58125 |
| Day 1 (n=3) | 0.66±0.58 | 0.33±0.58 |
| Day 3 (n=6) | 2.33±0.82 | 2.67±0.82 |
| Day 5 (n=8) | 4.43±0.79 | 4.13±0.64 |

Grades (0 – 6) correspond to the ISHLT classification.
the cardiac allografts. In a report from Koglin et al and in unpublished experiments in our laboratory, there was also reduced myocardial inflammation in cardiac allografts transplanted into iNOS-deficient mice in comparison to that seen in wild type control recipients. In the present study, there were slight but not significant reductions in the myocardial inflammation in the animals treated with the selective COX-2 and iNOS inhibitor drugs. Modest reductions in the degree of inflammation and the magnitude of the inflammatory infiltrate have also been reported following administration of SC-58125 to rats with adjuvant arthritis and to rats with carragecin-induced pleurisy.

Apoptosis of cardiac myocytes and of infiltrating macrophages has also been observed in parallel with the upregulation of iNOS mRNA, protein, and enzyme activity in rejecting rat cardiac allografts and in human endomyocardial biopsies from hearts undergoing class 3 (ISHLT) rejection. These associations, along with in vitro studies of NO-mediated cardiomyocyte apoptosis, have suggested that NO may be an apoptotic trigger in this situation. The slight reductions observed in apoptotic cell numbers in the animals treated with the COX-2 and iNOS inhibitors were not statistically significant. Recently, however, Koglin et al reported that during cardiac allograft rejection in iNOS knockout mice, the number of apoptotic cells was significantly reduced. It is of interest that von Knethen and Brune, in studies of NO-mediated apoptosis of RAW 264.7 macrophages in vitro, developed strong evidence that COX-2 is an essential regulator of apoptosis.

In summary, COX-2 mRNA and enzyme protein are upregulated in parallel with iNOS during cardiac allograft rejection. Although in this experimental model allograft survival was prolonged slightly, myocardial inflammation and cardiomyocyte apoptosis were not significantly reduced by treatment with a combination of inhibitors of COX-2 and iNOS.

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