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Interleukin-1 Receptor Type I Signaling Critically Regulates Infarct Healing and Cardiac Remodeling

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The proinflammatory cytokine interleukin (IL)-1 signals exclusively through the type I IL-1 receptor (IL-1RI). IL-1 expression is markedly induced in the infarcted heart; however, its role in cardiac injury and repair remains controversial. We examined the effects of disrupted IL-1 signaling on infarct healing and cardiac remodeling using IL-1RI^{-/-} mice. After reperfused infarction IL-1RI-null mice exhibited decreased infiltration of the infarcted myocardium with neutrophils and macrophages and reduced chemokine and cytokine expression. In the absence of IL-1 signaling, suppressed inflammation was followed by an attenuated fibrotic response. Infarcted IL-1RI^{-/-} mice had decreased myofibroblast infiltration and reduced collagen deposition in the infarcted and remodeling myocardium. IL-1RI deficiency protected against the development of adverse remodeling; however, infarct size was comparable between groups suggesting that the beneficial effects of IL-1RI gene disruption were not attributable to decreased cardiomyocyte injury. Reduced chamber dilation in IL-1RI-null animals was associated with decreased collagen deposition and attenuated matrix metalloproteinase (MMP)-2 and MMP-3 expression in the peri-infarct area, suggesting decreased fibrotic remodeling of the noninfarcted heart. IL-1 β stimulated MMP mRNA synthesis in wild-type, but not in IL-1RI-null cardiac fibroblasts. In conclusion, IL-1 signaling is essential for activation of inflammatory and fibrogenic pathways in the healing infarct, playing an important role in the pathogenesis of remodeling after infarction. Thus, interventional therapeutics targeting the IL-1 system may have great benefits in myocardial infarction. (*Am J Pathol* 2008, 173:57–67; DOI: 10.2353/ajpath.2008.070974)

en-based scar.¹ The healing response is closely intertwined with ventricular remodeling, a complex process that involves both the infarcted and noninfarcted myocardium resulting in dilation, hypertrophy, and enhanced sphericity of the ventricle.² The extent of remodeling after infarction is an important predictor of mortality and adverse outcome after infarction,^{3,4} and depends on the size of the infarct and on the mechanical and structural characteristics of the healing wound. Inflammatory mediators may be critically involved in the pathogenesis of cardiac remodeling by modulating cell behavior in the infarcted heart and by regulating extracellular matrix metabolism.^{5–7}

Interleukin (IL)-1 plays a central role in regulating inflammatory and fibrotic responses by inducing synthesis of proinflammatory mediators, by promoting leukocyte infiltration and activation, and by modulating fibroblast function. IL-1 binds to two distinct receptors on the cell membrane: the type I IL-1 receptor (IL-1RI) is sufficient to mediate all IL-1 actions,⁸ whereas the type II receptor (IL-1RII) serves as a decoy target,⁹ trapping and scavenging IL-1 molecules,¹⁰ thus reducing IL-1 concentration available for interaction with the IL-1RI signaling receptor.

Although several studies have demonstrated that IL-1 β is markedly induced in healing infarcts,^{11,12} the role of IL-1 signaling in myocardial infarction remains poorly understood and controversial. Administration of an anti-IL-1 β neutralizing antibody in the acute phase of non-reperfused murine myocardial infarction was detrimental, resulting in reduced collagen accumulation in the scar and attenuated adverse remodeling.¹³ In contrast, inhibition of IL-1-mediated effects through overexpression of IL-1R antagonist (IL-1Ra) decreased cardiomyocyte apoptosis and reduced inflammation decreasing myocardial injury after reperfused infarction.¹⁴ Our study investigates for the first time the effects of disruption of IL-1 signaling on infarct healing and cardiac remodeling after infarction. We found that IL-1RI-null mice exhibit attenuated ventricular dilation after reperfused infarction. The

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Infarct healing is dependent on induction of an inflammatory cascade that ultimately results in formation of a collagen-

protective effects of disrupted IL-1 signaling on the remodeling heart are not attributable to decreased cardiomyocyte injury, but are associated with a markedly suppressed cardiac inflammatory response and reduced fibrosis of the noninfarcted myocardium. The IL-1 system may be a promising therapeutic target for patients with myocardial infarction.

Materials and Methods

Murine Ischemia/Reperfusion Protocols

All animal studies were approved by the animal protocol review committee at Baylor College of Medicine. IL-1RI^{-/-} mice¹⁵ and WT C57/BL/6 controls (purchased from The Jackson Laboratories, Bar Harbor, ME) were used for myocardial infarction experiments. Female mice, 8 to 12 weeks of age (18.0 to 22.0 g body weight) were anesthetized by an intraperitoneal injection of sodium pentobarbital (60 μ g/g). A closed-chest mouse model of reperfused myocardial infarction was used to avoid the confounding effects of surgical trauma and inflammation, which may influence the baseline levels of chemokines and cytokines.¹⁶ The left anterior descending coronary artery was occluded for 1 hour then reperfused for 6 hours to 7 days. At the end of the experiment, the chest was opened and the heart was immediately excised, fixed in zinc-formalin, and embedded in paraffin for histological studies, or snap-frozen and stored at -80°C for RNA isolation. Sham animals were prepared identically without undergoing coronary occlusion/reperfusion. Animals used for histology underwent 24-hour, 72-hour, and 7-day reperfusion protocols (eight animals per group). Mice used for RNA extraction underwent 6 hours, 24 hours, and 72 hours of reperfusion (eight animals per group). To examine matrix metalloproteinase (MMP) and TIMP mRNA expression in the infarcted and remodeling myocardium hearts from animals undergoing 1 hour of ischemia and 7 days of reperfusion were used (eight mice per group). mRNA was extracted separately from the infarct and the remote noninfarcted myocardium. Additional animals [knockout (KO), $n = 10$; wild type (WT), $n = 8$] were used for perfusion-fixation after 7 days of reperfusion to assess remodeling-associated parameters.

Immunohistochemistry and Quantitative Histology

Murine hearts were fixed in zinc-formalin (Z-fix; Anatech, Battle Creek, MI), and embedded in paraffin. Sections were cut at 5 μ m and stained immunohistochemically with the following antibodies: anti- α smooth muscle actin (α -SMA) antibody (Sigma, St. Louis, MO), rat anti-mouse macrophage antibody Mac-2 (Cedarlane, Burlington, Canada), and rat anti-neutrophil antibody (Serotec, Raleigh, NC). Staining was performed using a peroxidase-based technique with the Vectastain ELITE rat, rabbit, or goat kit (Vector Laboratories, Burlingame, CA) and developed with diaminobenzidine plus nickel (Vector Lab-

oratories).¹⁷ The mouse on mouse (MOM) kit (Vector Laboratories) was used for α -SMA immunohistochemistry.¹⁶ Quantitative assessment of neutrophil and macrophage density was performed by counting the number of neutrophils and Mac-2-immunoreactive cells, respectively, in the infarcted area.¹⁶ Myofibroblasts were identified as extravascular α -SMA-positive cells and counted in the infarcted myocardium.⁶ Macrophage, neutrophil, and myofibroblast density was expressed as cells/mm². The collagen network was identified using Picrosirius red staining.¹⁶ Picrosirius red-stained slides from each infarcted heart were scanned using a digital camera. The percentage of the collagen-stained area was assessed in the infarcted myocardium, peri-infarct area, and remote remodeling myocardium using ImagePro software (Media Cybernetics, Bethesda, MD). Ten distinct fields from two different sections were used for quantitative analysis of collagen content in each area.

Perfusion Fixation and Assessment of Ventricular Volumes

For assessment of remodeling after infarction, infarcted hearts after 7 days of reperfusion were used for perfusion-fixation as previously described.¹⁶ The entire heart from base to apex was cross-sectioned at 250- μ m intervals. Ten serial 5- μ m sections were obtained at each interval. The first section from each interval was stained with hematoxylin and eosin and was used for morphometric assessment of left ventricular volumes and scar size. The left ventricular end-diastolic volume (LVEDV), left ventricular volume, septal volume, and scar size were assessed with ImagePro software using methods developed in our laboratory.^{18–20} The size of the infarct was expressed as a percentage of the left ventricular volume.

Echocardiography

Short axis M-mode echocardiographic studies were performed before instrumentation and after 7 days of reperfusion (WT, $n = 7$; IL-1RI^{-/-}, $n = 8$) using an 8 MHz probe (Sequoia C256; Acuson, Mountain View, CA). The following parameters were measured as indicators of function and remodeling: left ventricular end-diastolic diameter (LVEDD), left ventricular end-systolic diameter (LVESD), and fractional shortening ($\text{FS} = [\text{LVEDD} - \text{LVESD}] \times 100/\text{LVEDD}$). The percent change in these parameters after infarction was quantitatively assessed using the following formulas: $\Delta\text{LVEDD} = (\text{LVEDD 7 days} - \text{LVEDD pre}) \times 100/\text{LVEDD pre}$, $\Delta\text{LVESD} = (\text{LVESD 7 days} - \text{LVESD pre}) \times 100/\text{LVESD pre}$, $\Delta\text{FS} = (\text{FS pre} - \text{FS 7 days}) \times 100/\text{FS pre}$.

RNA Extraction and Ribonuclease Protection Assay

Inflammatory gene expression in murine hearts was assessed using a ribonuclease protection assay as

Table 1. Assessment of Echocardiographic Parameters in the Infarcted Heart

<i>n</i> = 14	WT pre	WT 168h	IL1RKO pre	IL1RKO 168h
LVEDD	3.37 ± 0.057	3.74 ± 0.067*	3.35 ± 0.054	3.56 ± 0.05*†
LVESD	1.97 ± 0.052	2.42 ± 0.066*	1.93 ± 0.067	2.32 ± 0.064*
FS	0.42 ± 0.139	0.35 ± 0.010*	0.41 ± 0.016	0.35 ± 0.013*
ΔLVEDD (%)		10.98 ± 2.247	6.601 ± 1.168 (<i>P</i> = 0.09)	
ΔFS (%)		17.79 ± 3.470	17.2 ± 3.507	

**P* < 0.01 versus corresponding pre.

†*P* < 0.01 versus corresponding WT.

previously described.^{12,16} The mRNA expression level of the chemokines MIP-1 α , MIP-1 β , MIP-2, MCP-1, and interferon- γ -inducible protein (IP)-10; the cytokines tumor necrosis factor (TNF)- α , IL-1 β , IL-6, and IL-10; the growth factors transforming growth factor (TGF)- β 1, -2, and -3, and M-CSF; the MMPs MMP-2, -3, -8, -9; and the TIMPs, TIMP-1, -2, -3, and -4 were determined using a ribonuclease protection assay (RiboQuant; Pharmingen, Franklin Lakes, NJ) according to the manufacturer's protocol. Phosphorimaging of the gels was performed (Storm 860; Molecular Dynamics, Sunnyvale, CA) and signals were quantified using Image QuANT software and normalized to the ribosomal protein L32 mRNA.

Protein Extraction and Western Blotting

Protein was isolated from the infarct and the noninfarcted remodeling myocardium of WT and IL-1RI-null hearts after 7 days of reperfusion. Western blotting was performed using a goat anti-MMP-2 and a rat anti-MMP-3 antibody (both from R&D Systems, San Diego, CA) as previously described.⁶

Cardiac Fibroblast Isolation and Stimulation

Fibroblasts were isolated from murine WT and IL-1RI-null hearts, cultured as previously described,^{21,22} and stimulated with rIL-1 β (10 ng/ml) (R&D Systems) for 2 to 16 hours. At the end of the experiment total RNA was isolated from the fibroblast cell lysates. MMP-2, -3, -8, -9, and TIMP-1, -2, -3, and -4 mRNA expressions were assessed using a ribonuclease protection assay.

Statistical Analysis

Statistical analysis was performed using analysis of variance followed by *t*-test corrected for multiple comparisons (Student-Newman-Keuls). Data were expressed as mean $\pm$ SEM. Statistical significance was set at 0.05.

Results

IL-1RI Gene Disruption Did Not Affect Cardiac Homeostasis

IL-1RI^{-/-} and WT mice had comparable cardiac morphology. IL-1RI gene disruption did not affect macro-

phage density and the morphology of the extracellular matrix network in the mouse heart (not shown). Furthermore, in the absence of injury, IL-1RI and WT hearts had comparable cardiac function and chamber dimensions (Table 1).

IL-1RI-Null Animals Showed Decreased Mortality after Reperfusion

IL-1RI-null mice showed a trend toward decreased mortality after myocardial infarction (WT, 14.7%, versus IL-1RI^{-/-}, 6.1%; *P* = 0.09). WT and IL-1RI-null mice had comparable mortality during coronary occlusion (WT, 8.1%, versus IL-1RI^{-/-}, 6.1%; *P* = NS); however, IL-1RI-null mice showed significantly decreased mortality during reperfusion (WT, 7.2%, versus IL-1RI^{-/-}, 1.1%; *P* = 0.05). The mortality rate during the initial instrumentation surgery was comparable between groups (WT, 4.9%, versus IL-1RI^{-/-}, 5.8%; *P* = NS).

IL-1RI-Null Mice Exhibited Markedly Reduced Neutrophil Infiltration in the Infarcted Myocardium

WT infarcts showed intense neutrophil infiltration, peaking after 24 hours of reperfusion (Figure 1A). IL-1RI gene disruption markedly reduced neutrophil density after 24 hours (IL-1RI^{-/-}, 223.5 $\pm$ 38.92 cells/mm², versus WT, 1037 $\pm$ 55.32 cells/mm²; *P* < 0.001) and 72 hours (IL-1RI^{-/-}, 156.0 $\pm$ 25.28 cells/mm², versus WT, 629.1 $\pm$ 57.67 cells/mm²; *P* < 0.001) of reperfusion (Figure 1). Both IL-1RI-null and WT animals had almost complete resolution of the neutrophilic infiltrate after 7 days of reperfusion (Figure 1, C, F, and G).

IL-1RI Gene Disruption Delayed Infiltration of the Infarcted Myocardium with Macrophages

Macrophages were identified in the infarcted mouse heart using Mac-2 immunohistochemistry (Figure 2). In comparison with WT mice (Figure 2, A–C), IL-1RI-null animals had significantly lower macrophage density in the infarcted myocardium after 24 hours of reperfusion (Figure 2, D and G). However, peak macrophage density after 72 hours of reperfusion was comparable between groups (Figure 2, B, E, and G). Macrophage density in

Figure 1. IL-1RI-null mice exhibited markedly reduced infiltration of the infarcted myocardium with neutrophils. Immunohistochemical staining demonstrated intense infiltration of WT infarcts with neutrophils after 24 hours (A) and 72 hours (B) of reperfusion. IL-1RI^{-/-} animals had markedly decreased neutrophil infiltration after 24 hours (D) and 72 hours (E) of reperfusion. Both WT (C) and IL-1RI KO mice (F) showed complete resolution of the neutrophil infiltrate after 7 days of reperfusion. G: Quantitative analysis of neutrophil density in the healing infarct (**P < 0.01 versus corresponding WT mice).

both WT and IL-1RI-null infarcts significantly decreased after 7 days of reperfusion (Figure 2, C and F).

IL-1RI^{-/-} Mice Had Markedly Reduced Peak Chemokine Expression in the Infarcted Myocardium

WT animals exhibited marked induction of the chemokines MIP-1 β (Figure 3A), MIP-1 α (Figure 3B), MIP-2 (Figure 3C), and MCP-1 (Figure 3E) peaking after 6 hours of reperfusion. IP-10 mRNA expression was also induced after 6 hours of reperfusion and showed a second delayed peak after 72 hours of reperfusion (Figure 3D). IL-1RI^{-/-} animals had markedly reduced peak mRNA expression of the chemokines MIP-1 α (Figure 3B), MIP-1 β (Figure 3A), MIP2 (Figure 3C), and MCP-1 (Figure 3E) in the infarcted myocardium. In addition, disruption of IL-1 signaling in IL-1RI-null mice abrogated the early but not the late peak of IP-10 induction (Figure 3D).

IL-1RI-Null Animals Had Decreased Expression of Proinflammatory Cytokines in the Infarcted Myocardium

WT animals had marked induction of the proinflammatory cytokines TNF- α (Figure 4A), IL-6 (Figure 4B), IL-1 β (Figure 4C), and M-CSF (Figure 4E) in the infarcted myocardium, peaking after 6 hours of reperfusion. IL-1RI-null mice showed reduced peak IL-1 β , IL-6, and M-CSF mRNA expression in the infarcted heart (Figure 4). In contrast, TNF- α mRNA expression was comparable between groups. IL-1RI deficiency also attenuated mRNA expression of the inhibitory cytokine IL-10 (Figure 4D) in the infarcted myocardium.

IL-1RI Gene Disruption Resulted in Reduced Infiltration of the Infarcted Myocardium with Myofibroblasts

In the healing infarct, the inflammatory response is followed by activation of fibrogenic pathways and infiltration

Figure 2. IL-1RI-null animals had significantly decreased macrophage infiltration after 24 hours of reperfusion. Immunohistochemical staining with Mac2 identified macrophages infiltrating infarcted WT hearts after 24 hours (A), 72 hours (B), and 7 days (C) of reperfusion. D: IL-1RI-null mice had decreased macrophage infiltration after 24 hours of reperfusion. However, macrophage density in IL-1RI^{-/-} infarcts after 72 hours (E) and 7 days (F) of reperfusion was comparable with WT animals. G: Quantitative analysis of macrophage density in the infarcted heart (**P* < 0.05 versus corresponding WT mice).

of the infarcted myocardium with myofibroblasts.²³ In WT infarcts the density of infiltrating myofibroblasts (identified as spindle-shaped α -SMA-expressing cells) peaks after 72 hours of reperfusion (Figure 5A).¹² IL-1RI-null animals had significantly decreased peak myofibroblast infiltration in the infarcted myocardium after 72 hours of reperfusion (Figure 5, B and C). However, infarct myofibroblast density was comparable between IL-1RI^{-/-} and WT animals after 7 days of reperfusion (Figure 5C). TGF- β plays an important role in myofibroblast differentiation and activation.²⁴ Accordingly, we compared mRNA expression levels of TGF- β isoforms between IL-1RI and WT infarcts. Infarcted IL-1RI-null animals exhibited decreased TGF- β 1, - β 2, and - β 3 mRNA expression after 24 hours of reperfusion in comparison with WT mice (Figure 5, D–F).

IL-1RI-Null Mice Exhibited Decreased Collagen Deposition in the Infarct and the Remodeling Peri-Infarct Area

In the absence of injury IL-1RI^{-/-} mice showed no abnormalities in the cardiac collagen network. The healing response ultimately results in replacement of dead car-

diomyocytes with a collagen-based scar. Compared with WT animals (Figure 6A) IL-1RI-null mice had reduced collagen deposition in the scar (WT, $31.9 \pm 1.6\%$, versus IL-1RI-null, $24.8 \pm 2.8\%$; *P* < 0.01) and in the neighboring peri-infarct area (WT, 12.2 ± 1.6 , versus IL-1RI^{-/-}, 8 ± 1.1 ; *P* < 0.05) after 7 days of reperfusion (Figure 6, B and C). Collagen content in the remote myocardium was comparable between groups (WT, 6.2 ± 0.9 , versus IL-1RI^{-/-}, 3.0 ± 0.5 ; *P* = NS).

IL-1RI-Null Mice Showed Attenuated Dilative Remodeling after Myocardial Infarction

Ventricular remodeling after infarction in WT and IL-1RI-null animals was assessed using quantitative morphometry (Figure 7, A and D) and echocardiography (Figure 7, B and E). In the absence of injury, WT and IL-1RI-null hearts had comparable chamber dimensions (Figure 7, F and G; Table 1). After 7 days of reperfusion WT animals showed a marked increase in LVEDD (Figure 7F), LVESD (Table 1), and LVEDV (Figure 7G) reflecting dilative remodeling of the infarcted ventricle. Both morphometric and echocardiographic analysis demonstrated that IL-1RI-null animals had attenuated ventricular dilation after myocardial infarction,

Figure 3. IL-1RI^{-/-} mice exhibited markedly reduced chemokine up-regulation in the infarcted heart. WT mice showed marked early mRNA induction of the chemokines MIP-1β (A), MIP-1α (B), MIP-2 (C), and MCP-1 (E) in the infarcted myocardium. D: IP-10 induction showed a biphasic response. IL-1RI gene disruption prevented the early chemokine response in the infarcted heart suggesting a key role for IL-1 signaling in mediating chemokine synthesis after ischemia and reperfusion (****P* < 0.001, ***P* < 0.01, **P* < 0.05 versus corresponding WT mice).

showing significantly lower LVEDD and LVEDV (Figure 7, Table 1) than their WT littermates. Attenuated adverse re-modeling of the infarcted ventricle was not attributable to a difference in infarct size (IL-1RI^{-/-}, 16.1 ± 1.7%, versus

WT, 15.0 ± 1.3%; *P* = NS) (Figure 7C). WT and IL-1RI-null animals exhibited comparable deterioration in systolic function after 7 days of reperfusion (FS WT, 0.353 ± 0.01, versus IL-1RI^{-/-}, 0.346 ± 0.01; *P* = NS) (Table 1).

Figure 4. IL-1RI^{-/-} mice had decreased cytokine up-regulation in the infarcted heart. mRNA expression of the proinflammatory cytokines TNF-α (A), IL-6 (B), IL-1β (C), and M-CSF (E) peaked after 6 hours of reperfusion in WT mouse infarcts. IL-1RI KO mice exhibited decreased peak IL-6 (B), IL-1β (C), and M-CSF (E) mRNA expression. A: In contrast, TNF-α expression levels were comparable between groups. D: Furthermore, IL-1RI-deficient mice had significantly attenuated induction of the inhibitory cytokine IL-10 after 24 hours of reperfusion (**P* < 0.05, ***P* < 0.01 versus corresponding WT mice).

Figure 5. IL-1RI deficiency is associated with reduced myofibroblast infiltration and decreased TGF- β isoform expression in the infarcted heart. Myofibroblasts were identified as spindle-shaped, α -SMA-expressing cells, predominantly located in the border zone of WT (A) and IL-1RI-null (B) infarcts (arrows). C: Quantitative analysis demonstrated that peak myofibroblast density in the infarcted myocardium was significantly lower in IL-1RI-null animals. D–F: TGF- β is a key mediator involved in myofibroblast differentiation and fibrous tissue deposition. TGF- β 1 (D), TGF- β 2 (E), and TGF- β 3 (F) mRNA levels were significantly lower in infarcted IL-1RI KO hearts after 24 hours of reperfusion (* $P < 0.05$ versus corresponding WT mice).

Disrupted IL-1 Signaling Results in Decreased MMP-2 and MMP-3 mRNA and Protein Expression in the Infarcted Heart

Because MMPs are critically involved in remodeling of the infarcted ventricle, we examined mRNA expression of MMPs and their inhibitors in infarcted and remote remodeling areas of the heart after 7 days of reperfusion. IL-1RI KO mice exhibited significantly reduced MMP-2 mRNA levels in the infarcted and remodeling areas of the ventricle (Table 2). In addition, MMP-3 mRNA expression was lower in IL-1RI-null infarcts. Western blotting experiments demonstrated that IL-1RI deficiency was associated with reduced MMP-2 and MMP-3 protein levels in the infarcted myocardium. In contrast, TIMP-4 mRNA levels were higher in infarcted and noninfarcted IL-1RI cardiac segments (Table 2). MMP-8, MMP-9, TIMP-1,

and TIMP-2 mRNA expression was comparable between groups. The findings indicated that IL-1RI deficiency altered the MMP:TIMP expression profile in the infarct and the remote remodeling heart reducing MMP-2 and -3 expression.

IL-1 β Induces MMP Synthesis in Isolated Murine Cardiac Fibroblasts through an IL-1RI-Dependent Mechanism

IL-1 β stimulation significantly enhanced MMP-3, MMP-8, and MMP-9 mRNA synthesis and reduced TIMP-2 and TIMP-4 mRNA expression by isolated WT mouse cardiac fibroblasts (Table 3). However, fibroblast MMP-2, TIMP-1, and TIMP-3 mRNA expression was not significantly affected by IL-1 β stimulation. In

Figure 6. IL-1RI-null mice exhibited attenuated fibrotic remodeling of the infarcted ventricle. Picrosirius red staining was used to label the collagen network in WT (A) and IL-1RI-null (B) infarcts. Collagen content was quantitatively assessed in the infarct (arrowheads), the peri-infarct area (Peri-I, arrows), and the remote myocardium. C: IL-1RI KO mice had significantly lower collagen percent content in the infarct and the peri-infarct area (** $P < 0.01$, * $P < 0.05$ versus corresponding WT).

Figure 7. IL-1RI^{-/-} animals had attenuated adverse remodeling after myocardial infarction. Remodeling after infarction was assessed using morphometric (A: WT; D: IL-1RI^{-/-}) and echocardiographic techniques (B: WT; E: IL-1RI^{-/-}). Although WT and KO animals had comparable infarct size after 7 days of reperfusion (C), IL-1RI deficiency protected from the development of ventricular dilation. Both echocardiographically derived LVEDD (F) and morphometrically assessed LVEDV (G) were lower in infarcted IL-1RI-null mice 7 days after reperfusion in comparison with WT animals (***P* < 0.01 versus pre or sham, **P* < 0.05 versus corresponding WT).

contrast, fibroblasts isolated from IL-1RI-null mice did not respond to IL-1 β stimulation, exhibiting MMP and TIMP mRNA expression levels comparable with unstimulated fibroblasts (Table 3).

Discussion

Our findings demonstrate a critical role for IL-1-mediated interactions in the pathogenesis of adverse cardiac remodeling after myocardial infarction. Disruption of IL-1 signaling in genetically targeted IL-1RI-null mice protected from the development of ventricular dilation after

reperfused infarction. The protective effect of IL-1RI deficiency was not attributable to a reduction in the extent of cardiomyocyte injury, but was associated with a markedly suppressed inflammatory response, attenuated fibrous tissue deposition in the peri-infarct area and an altered MMP:TIMP expression profile.

IL-1 α and IL-1 β are pleiotropic cytokines that activate inflammatory pathways, signaling exclusively via the type I receptor.⁸ IL-1 β is markedly induced in the infarcted heart,^{11,12} and is rapidly released in the plasma of patients with acute myocardial infarction.²⁵ Our findings demonstrate that IL-1 signaling plays a key role in trig-

Table 2. MMP and TIMP Expression Profile in the Infarcted and Remote Remodeling Myocardium after 7 Days of Reperfusion

	WT infarct	KO infarct	WT remote	KO remote
MMP/TIMP mRNA (ratio to L32)				
MMP2	0.515 $\pm$ 0.022	0.388 $\pm$ 0.028*	0.179 $\pm$ 0.013	0.153 $\pm$ 0.0034*
MMP3	0.037 $\pm$ 0.004	0.024 $\pm$ 0.004*	0.016 $\pm$ 0.002	0.022 $\pm$ 0.002*
MMP8	0.024 $\pm$ 0.002	0.034 $\pm$ 0.005	0.028 $\pm$ 0.003	0.038 $\pm$ 0.006
MMP9	0.037 $\pm$ 0.004	0.028 $\pm$ 0.004	0.025 $\pm$ 0.002	0.025 $\pm$ 0.003
TIMP1	0.264 $\pm$ 0.0142	0.282 $\pm$ 0.030	0.150 $\pm$ 0.017	0.126 $\pm$ 0.016
TIMP2	0.395 $\pm$ 0.016	0.456 $\pm$ 0.036	0.350 $\pm$ 0.025	0.316 $\pm$ 0.021
TIMP3	0.310 $\pm$ 0.017	0.236 $\pm$ 0.028*	0.118 $\pm$ 0.017	0.268 $\pm$ 0.016†
TIMP4	0.024 $\pm$ 0.0054	0.051 $\pm$ 0.007†	0.086 $\pm$ 0.001	0.113 $\pm$ 0.008*
MMP protein (ratio to GAPDH)				
MMP-2	0.95 $\pm$ 0.12	0.61 $\pm$ 0.06†	0.21 $\pm$ 0.04	0.14 $\pm$ 0.02
MMP-3	1.05 $\pm$ 0.16	0.6 $\pm$ 0.08†	0.68 $\pm$ 0.06	0.35 $\pm$ 0.03

**P* < 0.05 and †*P* < 0.01 versus corresponding WT.

Table 3. Effects of IL-1 β Stimulation (10 ng/ml) on MMP and TIMP mRNA Expression by Isolated WT and IL-1RI-Null Cardiac Fibroblasts (-Fold Increase Versus Corresponding Control Unstimulated Fibroblasts)

	2 hours		6 hours		16 hours	
	WT	-/-	WT	-/-	WT	-/-
MMP-2	1.06 $\pm$ 0.19	0.84 $\pm$ 0.11	1.16 $\pm$ 0.12	0.73 $\pm$ 0.13	1.40 $\pm$ 0.17	0.81 $\pm$ 0.13
MMP-3	1.69 $\pm$ 0.25	1.01 $\pm$ 0.06	3.47 $\pm$ 0.32 [†]	0.92 $\pm$ 0.10	5.74 $\pm$ 0.32 [†]	1.00 $\pm$ 0.08
MMP-8	1.22 $\pm$ 0.05	1.07 $\pm$ 0.08	1.85 $\pm$ 0.36	0.95 $\pm$ 0.06	3.98 $\pm$ 0.82 [†]	0.97 $\pm$ 0.06
MMP-9	1.59 $\pm$ 0.01*	0.87 $\pm$ 0.07	3.45 $\pm$ 0.13 [†]	0.92 $\pm$ 0.03	5.86 $\pm$ 0.12 [†]	0.88 $\pm$ 0.03
TIMP-1	0.96 $\pm$ 0.15	0.88 $\pm$ 0.18	0.80 $\pm$ 0.27	0.90 $\pm$ 0.17	0.70 $\pm$ 0.21	0.89 $\pm$ 0.17
TIMP-2	0.84 $\pm$ 0.07	1.14 $\pm$ 0.11	0.56 $\pm$ 0.12*	1.15 $\pm$ 0.02	0.54 $\pm$ 0.08 [†]	1.11 $\pm$ 0.20
TIMP-3	1.09 $\pm$ 0.24	0.91 $\pm$ 0.05	1.16 $\pm$ 0.73	0.76 $\pm$ 0.11	0.8 $\pm$ 0.28	0.83 $\pm$ 0.11
TIMP-4	0.63 $\pm$ 0.02 [†]	0.88 $\pm$ 0.07	0.58 $\pm$ 0.03 [†]	0.84 $\pm$ 0.17	0.45 $\pm$ 0.01 [†]	0.98 $\pm$ 0.14

* $P < 0.05$ and [†] $P < 0.01$ versus corresponding control.

gering the inflammatory cascade in the infarcted myocardium; these observations are consistent with a previous investigation demonstrating an attenuated inflammatory reaction in IL-1RI-null animals after hepatic ischemia.²⁶ Peak expression of cytokines and chemokines was significantly decreased in IL-1RI-null myocardial infarcts (Figures 3 and 4). Furthermore, IL-1RI gene disruption markedly reduced neutrophil infiltration and delayed macrophage recruitment in the infarcted myocardium (Figures 1 and 2). The greatly diminished neutrophil density in IL-1RI-null infarcts may reflect both decreased recruitment of neutrophils and their increased susceptibility to apoptosis. IL-1 strongly prolongs neutrophil survival by inhibiting their apoptotic death.²⁷

Suppressed inflammation in ischemic IL-1RI-null hearts was not associated with less extensive infarction, suggesting that endogenous IL-1 does not exacerbate cardiomyocyte injury. Suzuki and co-workers¹⁴ have previously demonstrated that IL-1Ra overexpression by gene transfection resulted in reduced cardiomyocyte apoptosis and protection from ischemic injury in heterotopically transplanted rat hearts undergoing coronary occlusion and reperfusion. Although our findings also support a beneficial effect of disrupted IL-1 signaling in healing infarction, the mechanisms of protection do not involve attenuation of ischemic cardiomyocyte injury.

The suppressed inflammatory reaction in IL-1RI-null infarcts was followed by an attenuated fibrotic response. Myofibroblast accumulation in the infarcted area was significantly lower in IL-1RI^{-/-} infarcts in comparison with WT animals (Figure 5). In addition, expression of the key profibrotic mediator TGF- β ²⁸ was significantly reduced, and collagen deposition was markedly decreased, in both the healing scar and the peri-infarct area of IL-1RI^{-/-} hearts (Figures 5 and 6). In the absence of IL-1 signaling, reduced fibrotic remodeling of the infarcted ventricle may be attributable to an attenuated inflammatory reaction and to the loss of direct stimulatory IL-1-mediated effects on cardiac fibroblast phenotype and function. IL-1 β directly enhances fibrous tissue deposition by up-regulating expression of angiotensin II type 1 (AT1) receptors on cardiac fibroblasts²⁹ and by stimulating fibroblast migration.³⁰

Beyond its proinflammatory and fibrogenic properties, IL-1 also promotes extracellular matrix remodeling by enhancing cardiac fibroblast MMP expression.³¹ We

found that IL-1 β stimulation induced MMP-3, MMP-8, and MMP-9 mRNA synthesis by isolated mouse cardiac fibroblasts, while down-regulating TIMP-2 and TIMP-4 expression levels (Table 3). In the complex and dynamic environment of the infarct, where cellular behavior is regulated by a variety of mediators, the contribution of direct IL-1-mediated actions on fibroblast protease expression and extracellular matrix remodeling is difficult to assess. In comparison with WT animals, IL-1RI-null mice exhibited a decrease in MMP-2 and MMP-3 expression in both the infarcted and remote remodeling myocardium, supporting the *in vivo* relevance of IL-1-mediated effects on synthesis of matrix-degrading proteases.

Our experiments demonstrated that the cellular and molecular alterations observed in IL-1RI-null infarcts result in significant attenuation of dilative remodeling after infarction (Figure 7, Table 2). Protection from adverse remodeling in the absence of IL-1 signaling was not attributable to enhanced cardiomyocyte survival. Despite the marked suppression of the inflammatory response after infarction in infarcted IL-1RI-null animals, infarct size was comparable with WT mice, suggesting that IL-1-mediated inflammatory activity does not accentuate ischemic injury. However, suppression of inflammation may be protective by reducing fibrotic remodeling of the infarcted ventricle. Decreased interstitial fibrosis and reduced MMP synthesis in the remodeling noninfarcted myocardium of IL-1RI-null hearts may indicate attenuated interstitial remodeling. Fibrosis is often associated with enhanced matrix degradation indicating active remodeling of the interstitial space.³² IL-1RI gene disruption appears to abrogate both events resulting in decreased activity of the remodeling interstitial space and attenuated ventricular dilation. The beneficial effects of defective IL-1 signaling in remodeling after infarction may be mediated both through suppression of the inflammatory response and through the loss of direct IL-1-mediated effects on cardiac fibroblasts.

Our investigation identifies the IL-1 signaling pathway as a potential therapeutic target for the treatment of patients with acute myocardial infarction. Disruption of IL-1 signaling prevents the development of maladaptive fibrosis after myocardial infarction resulting in attenuated adverse remodeling. Despite the suppression of the inflammatory response after infarction, clearance of the infarct from dead cardiomyocytes occurs in a timely manner and

a collagen-based scar is formed. Although our experiments demonstrate that IL-1RI signaling is deleterious for the infarcted heart, studies using IL-1 inhibition strategies in experimental models of myocardial infarction have produced contradictory results.^{13,14} IL-1Ra overexpression in a model of reperfused infarction protected the heart by attenuating the inflammatory response.¹⁴ Moreover, transplantation of skeletal myoblasts secreting sIL-1Ra into the infarct border zone significantly reduced cardiomyocyte hypertrophy and interstitial fibrosis decreasing dilative remodeling.³³ In contrast, early IL-1 β inhibition in a model of nonreperfused infarction through injection with a neutralizing antibody resulted in worse remodeling of the infarcted heart.¹³ Several important considerations may explain the contradictory findings. First, selective inhibition of specific inflammatory mediators may be more effective in reperfused infarcts, which exhibit early and intense activation of inflammatory pathways. In contrast, nonreperfused infarcts show delayed and suppressed inflammation; IL-1 inhibition in this context may critically impair the healing response. Second, timing of the intervention is a key determinant of outcome. IL-1 is a highly pleiotropic factor that exerts distinct effects on many different cell types involved in all phases of the healing response. Early inhibition of IL-1 signaling is more likely to inhibit the inflammatory cascade, whereas late inhibition may predominantly abrogate the direct actions of IL-1 on fibroblasts. Third, the spatial localization of the inhibitory strategy may critically affect the outcome. Selective inhibition of inflammatory mediators in the infarct border zone and the remodeling myocardium may contribute to effective containment of the inflammatory response after infarction, reducing fibrotic remodeling and attenuating chamber dilation. In contrast, interventions selectively targeting the infarcted area are likely to be less predictable because excessive inhibition of the inflammatory response may result in formation of a defective scar.

Although our study demonstrates a critical role for IL-1RI signaling in the pathogenesis of remodeling after infarction, the specific mechanism responsible for the protection afforded by abrogation of IL-1 signaling remains unknown. Experiments using animals with conditional inactivation of IL-1RI in macrophages and fibroblasts may elucidate the cell biology of IL-1-mediated interactions in the healing infarct.

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