IRAK1 deletion disrupts cardiac Toll/IL-1 signaling and protects against contractile dysfunction

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IRAK1 deletion disrupts cardiac Toll/IL-1 signaling and protects against contractile dysfunction. Am J Physiol Heart Circ Physiol 285: H597–H606, 2003. First published April 24, 2003; 10.1152/ajpheart.00655.2001.—Myocardial contractile dysfunction accompanies both systemic and cardiac insults. Septic shock and burn trauma can lead to reversible contractile deficits, whereas ischemia and direct inflammation of the heart can precipitate transient or permanent impairments in contractility. Many of the insults that trigger contractile dysfunction also activate the innate immune system. Activation of the innate immune response to infection is coordinated by the conserved Toll/interleukin-1 (IL-1) signal transduction pathway. Interestingly, components of this pathway are also expressed in normal and failing hearts, although their function is unknown. The hypotheses that Toll/IL-1 signaling occurs in the heart and that intact pathway function is required for contractile dysfunction after different insults were tested. Results from these experiments demonstrate that lipopolysaccharides (LPS) activate Toll/IL-1 signaling and IL-1 receptor-associated kinase-1 (IRAK1), a critical pathway intermediate in the heart, indicating that the function of this pathway is not limited to immune system tissues. Moreover, hearts lacking IRAK1 exhibit impaired LPS-triggered downstream signal transduction. Hearts from IRAK1-deficient mice also resist acute LPS-induced contractile dysfunction. Finally, IRAK1 inactivation enhances survival of transgenic mice that develop severe myocarditis and lethal heart failure. Thus the Toll/IL-1 pathway is active in myocardial tissue and interference with pathway function, through IRAK1 inactivation, may represent a novel strategy to protect against cardiac contractile dysfunction.

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CARDIAC CONTRACTILE dysfunction, the impaired ability of the heart to contract or relax in response to different inotropic stimuli, accompanies many disorders and can be transient, permanent, or progressive. It occurs in diseases affecting primarily the heart and as a complication of conditions affecting other organs or tissues. In some conditions, such as septic shock or large body surface area burns, myocardial depression is reversible if the host survives (2, 31). After other insults, as with myocardial infarction, contractile dysfunction may consist of both transitory and permanent components. Stunning eventually resolves (17), but the loss of sufficient viable myocardium results in irreversibly impaired contractility. Heart failure, in contrast, is a progressive, lethal form of contractile dysfunction, with an overall 5-yr mortality ranging from 45% to 75% (22).

Three types of observations have linked myocardial dysfunction with inflammation. First, acute infections and inflammatory diseases can precipitate transient heart failure (29–32, 39). Second, both microbial and endogenous proinflammatory mediators, such as bacterial endotoxin [lipopolysaccharide (LPS)], interleukin-1 (IL-1), and tumor necrosis factor (TNF)-α directly depress myocardial contractility (5, 9, 21, 35, 37, 38). Third, elevated circulating concentrations of these molecules also occur in patients with heart failure of any cause and are correlated with more severe myocardial dysfunction (18, 27, 46).

Expression of the Toll-like receptor-4 (TLR4), the mammalian LPS sensor, together with its intracellular signaling apparatus (6, 7, 10, 13, 23, 33) in the heart, suggests a mechanism whereby LPS could trigger cardiac dysfunction. In immune cells such as macrophages, LPS signals through an intracellular pathway shared with the type 1 IL-1 receptor (the Toll/IL-1 pathway). After activation of TLR4 or the type 1 IL-1 receptor, MyD88 and IL-1 receptor associated kinase-1 (IRAK1) are recruited to the activated receptor complex (6, 24, 48). IRAK1 becomes phosphorylated, dissociates from the receptor complex, and associates with TNF receptor-associated factor 6 (6, 7). The signal is then distributed to multiple downstream targets, including NF-κB, c-Jun NH2-terminal kinase (JNK), and...
p38α MAPK. Deletion of MyD88, IRAK1, or TNF receptor-associated factor 6 disrupts both IL-1 and LPS signaling to distal pathways and disturbs normal physiological responses to these proinflammatory mediators in innate immune cells (1, 15, 16, 20, 25, 41, 44).

The presence of Toll/IL-1 signaling molecules in the heart and their upregulation in disease states such as congestive heart failure led to the hypothesis that the same pathway that initiates the inflammatory response to infection in immune cells also functions in the heart and mediates acute and chronic myocardial contractile dysfunction. Although previous studies (26, 45) have demonstrated a role for TLR4 in both LPS- and burn-induced myocardial dysfunction, a requirement for IRAK1 has only been shown in burn-triggered contractile impairment (43). Thus it is unknown whether TLR4 stimulation activates IRAK1 in the heart or whether IRAK1 function is necessary for contractile dysfunction after LPS administration. Studies reported here show that LPS activates IRAK1 in the heart. Moreover, IRAK1 inactivation alters cardiac signaling responses to LPS and protects mice from LPS-induced contractile function. Finally, IRAK1 deletion enhances survival in transgenic mice with myocarditis, cardiomyopathy, and lethal heart failure. Thus interference with Toll/IL-1 signaling, as occurs with IRAK1 inactivation, may represent a therapeutic strategy to ameliorate conditions associated with either acute or chronic cardiac contractile dysfunction.

MATERIALS AND METHODS

Reagents. Escherichia coli LPS (O111:B4, Sigma) was used for in vivo injections. Antisera against IRAK1 and JNK were provided by Zhaodan Cao (Tularik) and Melanie Cobb (University of Texas Southwestern), respectively. Polyclonal antisera against IKK-β and IκB-α were purchased from Santa Cruz Biotechnology and New England Biolabs, respectively. Recombinant glutathione S-transferase-IκB-α and glutathione S-transferase-c-Jun were provided by Melanie Cobb.

Animals. All animals were used in compliance with the guidelines established by the Institutional Animal Care and Research Advisory Committee at the University of Texas Southwestern Medical Center and performed in accordance with National Institutes of Health guidelines for the use of laboratory animals.

IRAK1-deficient and cardiac-specific TNF-α overexpressor mice were generated and genotyped as previously described (4, 44). For generation of the IRAK1 knockout (KO), the Irak1 gene was inactivated in murine embryonic stem cells using targeted mutagenesis. Mutant stem cells were injected into wild-type (WT) blastocysts and resulted in chimeric mice. Two chimeric animals transmitted the mutant Irak1 gene through the germline. Offspring from these founders were interbred to generate homozygous IRAK1-deficient females and hemizygous IRAK1-deficient males (Irak1 is located on the X chromosome) (44). These animals were on a hybrid background (129Sv × C57BL/6). WT animals on a similar hybrid background were used as controls for all experiments. Cardiac-specific TNF-α-overexpressing mice were generated by microinjection of a transgene encoding the murine TNF-α sequence coupled to an α-myosin heavy chain promoter into the male pronucleus of fertilized mouse eggs and implanted into pseudopregnant females. Offspring with the integrated transgene became founders for this novel line of transgenic mice. To generate transgenic KO double mutant mice, homozygous IRAK1-deficient females were crossed to male TNF-α overexpressors. Male transgenic offspring were crossed to IRAK1 KO females and progeny with the TNF-α transgene were used in subsequent studies. Hybrid (C57Bl/6 J × SJL) WT mice also served as additional controls.

In vitro kinase reactions. At different times after LPS injection, mice were euthanized, and the hearts were immediately removed and snap frozen in liquid nitrogen. Longitudinal sections (25–40 mg) that included both atrial and ventricular tissue were cut from frozen hearts, rinsed in ice-cold phosphate-buffered saline to remove clotted blood, and minced into small pieces using a number 11 scalpel blade. The tissue was then disrupted in a Dounce homogenizer with 1 ml of either a HEPES lysis buffer (50 mM HEPES, pH 7.5; 150 mM NaCl; 1% Triton X-100; 10% glyc erol, and 1 mM dithiothreitol (DTT)) with protease (Complete Inhibitor, Roche; Indianapolis, IN) and phosphatase inhibitors (20 mM NaF, 20 mM sodium glycerophosphate, and 0.5 mM sodium orthovanadate) for all kinases except IRAK1, or a RIPA lysis buffer (50 mM Tris·HCl, pH 7.4; 150 mM NaCl; 1% Nonidet P-40; 0.25% sodium deoxycholate; 0.1% SDS; and 1 mM DTT) with the same protease and phosphatase inhibitors. After a 30-min incubation on ice, lysates were cleared by centrifugation at 20,000 g for 10 min at 4°C, and the supernatant was transferred to a clean microcentrifuge tube. The total protein concentration of the lysates was determined with the use of a protein assay kit (Bio-Rad; Hercules, CA). Kinases were immunoprecipitated from 1 mg of total cardiac protein in a minimum 250-μl volume with 1 to 5 μl of antisera on rocking platform overnight at 4°C. Protein A-conjugated agarose (20 μl; Roche) was added and allowed to incubate for 2 h at 4°C. Precipitates were washed three times with lysis buffer and once with incomplete kinase buffer composed of (in mM) 50 Tris·HCl (pH 7.4), 10 MgCl2, and 1 DTT. Beads were then resuspended in complete kinase buffer composed of 50 mM Tris·HCl (pH 7.4), 10 mM MgCl2, 1 mM DTT, 50 μM “cold” ATP, and [γ-32P]ATP (10 μCi/reaction) together with appropriate substrate (0.3 mg/ml) in a total volume of 25 μl. The mixtures were incubated at 30°C for 30 min. Reactions were stopped by adding an equal volume of 2× SDS-PAGE sample buffer (200 mM Tris-HCl, 4% SDS, 0.04% bromophenol blue, and 20% glycerol), followed by heating at 95°C for 5 min. Samples were fractionated on a large-format 10% SDS-polyacrylamide gel and the proteins were transferred to a polyvinylidene difluoride membrane (Immobilon-P, Millipore; Bedford, MA). Membranes were air dried, exposed to phosphor storage screens, and developed with the use of a phosphorimaging system (Molecular Dynamics; Mountain View, CA). Fold catalytic activation was determined by densitometric increases in signal above the 0-h time point.

Electromobility shift assays. A modified procedure based on the method of Schreiber et al. (34) was used. Longitudinal heart sections (25 mg) were homogenized in ice-cold hypotonic lysis buffer composed of (in mM) 10 Tris·HCl (pH 7.8), 5 MgCl2, 10 KCl, 0.3 EGTA, and 0.5 DTT and 0.3 M sucrose (protease inhibitors) and incubated on ice for 15 min. After the addition of Nonidet P-40 (final concentration: 0.5%) the mixture was vortexed and centrifuged to recover nuclei. The supernatant was saved for IκB immunoblots. Nuclear proteins were extracted from the pellet that was composed of (in mM) 20 Tris·HCl (pH 7.8), 5 MgCl2, 320 KCl, 0.2 EGTA, and 0.5 DTT (plus protease inhibitors) for 15 min on ice and centrifuged at 13,500 g for 15 min. Extracts were stored at

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–80°C. Total protein concentration of nuclear extracts was determined with a protein assay kit (Bio-Rad).

Double-stranded oligonucleotide corresponding to the consensus NF-κB binding site of the murine κ-light enhancer (5′-AGTTGAGGGACTTCCAGGC-3′) was purchased from Amersham Pharmacia Biotech (Piscataway, NJ). Oligonucleotide (3.5 pmol), T4 polynucleotide kinase (5 units) in kinase buffer (Promega; Madison, WI), and [γ-32P]ATP (30 μCi; DuPont-New England Nuclear; Boston, MA) were incubated at 37°C for 60 min. The labeled probe was separated from unbound ATP with the use of microcolumns (ProbeQuant G-50, Amersham Pharmacia Biotech) and stored at –20°C. Nuclear proteins (2.5 μg) were incubated with 500,000 counts/min of probe in the presence of salmon sperm DNA (2 μg) in 1× gel shift buffer composed of (in mM) 20 HEPES, 50 KCl, 1 DTT, and 1 EDTA (pH 7.6) and 5% glycerol for 30 min at room temperature. The mixtures were then separated on a nondenaturing 8% polyacrylamide gel in 0.5× 25 mM Tris-HCl, 25 mM boric acid, and 0.5 mM EDTA. The gel was dried and exposed to X-ray film (Kodak BioMax).

**Immunoblotts.** Samples containing cytosolic protein (50 μg) were separated with the PE-50 tubing threaded into the ascending aorta and connected via glass tubing to a high-speed electronic differentiator (model 7P20C, Grass Instruments), and transferred to a Dell Pentium computer. Contractile function was determined by plotting LV systolic pressure and ±dP/dt max values against increases in coronary flow, achieved by increasing the flow rate of a pump calibrated to deliver between 1 and 4 ml/min into the cannulated aortic stump, or perfusate Ca2+ concentration. After removal, cardioplegia, cannulation, and perfusion, ex vivo denervated hearts typically beat between 280 and 310 beats/min. If the rhythm was too slow, too fast, or irregular, the hearts were captured at their intrinsic rates and then paced at ~300 beats/min through an electrode attached to the right atrium (4.8–5.0 A for 1-ms duration, Grass Stimulator) for the duration of the contractile function studies. There is no difference between groups with regard to the requirements for pacing.

**Histology.** Hearts were fixed by immersion in 10% neutral-buffered formalin, embedded in paraffin, and sectioned at 6 μm. Sections were stained with hematoxylin and eosin for routine examination and with Masson’s trichrome to reveal collagen. Coded samples were scored by a single veterinary pathologist (D. F. Kusewitt) located at another institution for severity of myocarditis on a scale of 0–3 (0, no myocarditis; 1, mild myocarditis; 2, moderate myocarditis; 3, severe myocarditis). The reader was blinded to the animals’ genotypes. Severity scores were based on the extent of myocardial involvement and the degree of interstitial cellularity and fibrosis in affected areas. Less than 25% involvement of the heart was scored as mild myocarditis; in cases of moderate myocarditis, 25–50% of the heart was affected; in cases of severe myocarditis, >50% of the heart was involved.

**Statistics.** Separate analyses of cardiac functions were conducted for each of LVP, ±dP/dt, and –dP/dt as a function of treatment group (factor 1: WT sham vs. LPS, KO sham vs. LPS, sham WT vs. KO) and either coronary flow rate or calcium level (factor 2). A two-way analysis of variance was performed. In those instances in which the factor 1–2 interaction was significant at the 0.05 level, Bonferroni-corrected post tests were then conducted at each level of factor 2 (coronary flow rate or perfusate calcium) to discern differences among the treatment groups. Survival curves of TNF-α transgenic and transgenic/IRAK1-deficient mice were compared using a Kaplan-Meier survival estimate.

**RESULTS**

**IRAK1 is activated in heart by LPS and is required for optimal downstream signaling.** To determine whether LPS activates IRAK1 in the heart, WT mice were challenged with LPS and IRAK1 catalytic activity was measured using an immunoprecipitation in vitro kinase assay. Thirty minutes after LPS treatment, IRAK1 catalytic activity increased about threefold to activation of two downstream signaling pathways in the heart was then examined. LPS can cause rapid cardiac NF-κB activation (14). The responses of hearts from WT and IRAK1-deficient mice (44) were then compared. Animals were injected with LPS (1 mg/kg ip), their hearts were removed, and NF-κB activation was examined at various times. Catalytic activation of IKK-β, the kinase that phosphorylates IkB-α and targets it for degradation, was assayed first. As shown in Fig. 1B, IKK-β catalytic activity increased rapidly in...
WT hearts, reached 15-fold activation after 30 min, and then declined, returning to baseline by 3 h. In contrast, IKK-β activity in IRAK1-deficient hearts was diminished and delayed compared with WT, with peak activation sevenfold above baseline. Similar results were obtained in two other trials. Thus IRAK1 governs both the kinetics and peak activity of the principle kinases controlling NF-κB activation in the heart, an effect similar to that seen in LPS-treated macrophages (41). This finding is also reflected by the delayed and incomplete LPS-induced IkB-α degradation (Fig. 1C) and reduced NF-κB DNA binding activity in IRAK1-deficient compared with WT hearts (Fig. 1D). Together, these findings demonstrate that LPS-mediated NF-κB activation in the heart operates through an IRAK1-dependent pathway.

The contribution of IRAK1 to JNK signaling in the heart was also examined. JNK activity controls LPS-induced TNF-α production in macrophages (12, 40). This cytokine is produced in cardiac myocytes after LPS stimulation and causes myocardial contractile dysfunction (4, 5, 38). LPS treatment of WT mice triggered a 28-fold increase in cardiac JNK activity in the experiment shown in Fig. 1E. Peak activation that occurred 15 min after injection returned to basal levels by 60 min. IRAK1-deficient hearts, on the other hand, exhibited a severely attenuated and delayed response to LPS injection (Fig. 1E), demonstrating that IRAK1 is required for LPS-induced JNK activation in heart tissue.

IRAK1 mediates LPS-induced myocardial contractile dysfunction. Sepsis causes myocardial contractile dysfunction (30, 32), and administration of endotoxin to both humans and experimental animals mimics the myocardial depression of sepsis (30, 39). To determine whether differences in intracellular signaling were also associated with altered myocardial contractile function, the contractile responses of hearts from LPS-treated WT and IRAK1-deficient mice were compared. Animals were injected with either LPS (1 mg/kg ip) or an equal volume of normal saline. Eighteen hours later, the hearts were removed and contractile function was assessed ex vivo with the use of modified Langendorff-isolated perfusion preparations. There was no difference in baseline contractile function between hearts from WT or IRAK1-deficient mice. Both exhibited similar systolic maximal developed LVP and rate of pressure generation (+dP/dt_max) and diastolic rate of relaxation (−dP/dt_max) responses to increasing coro-

![Image](http://ajpheart.physiology.org/)

**Fig. 1.** Lipopolysaccharide (LPS) activation of intracardiac signaling pathways. A: LPS induction of interleukin-1 receptor-associated kinase-1 (IRAK1) catalytic activity in the heart. IRAK1 was immunoprecipitated (IP) from cardiac tissue of wild-type (WT) saline- or LPS (1 mg/kg ip)-treated mice and incubated in the presence of [γ-32P]ATP and myelin basic protein (MBP). B: impaired IKK-β activation in IRAK1−/− (knockout; KO) hearts. Hearts from LPS-treated WT and KO mice were assayed for IKK-β kinase activity using an IP kinase assay with recombinant IkB-α as the substrate. C: delayed and incomplete IkB-α degradation in IRAK1-deficient hearts. Equal amounts of protein from WT and KO heart cytoplasmic extracts were immunoblotted with a commercially available antiserum against IkB-α. WB, Western blot. D: optimal nuclear factor-κB (NF-κB) activation requires IRAK1. Nuclear extracts were incubated with a radioactively labeled oligonucleotide containing an NF-κB consensus binding sequence and fractionated on a native polyacrylamide gel. E: requirement for IRAK1 in LPS induction of cardiac c-Jun NH2-terminal kinase (JNK) activation. Hearts from LPS-treated WT and KO mice assayed for JNK catalytic activity with an IP kinase assay using recombinant c-Jun as substrate. GST, glutathione S-transferase. Extracts used for assays in B–E come from the same hearts. Data shown are from one of three representative experiments.

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nary flow rates (Fig. 2) and perfusate calcium concentrations (Fig. 3A). As expected, WT LPS-treated hearts exhibited significant myocardial contractile dysfunction compared with sham-treated WT hearts. This impaired function affects both systole and diastole and persists at increasing coronary flow rates (Fig. 2) and extracellular calcium concentrations (Fig. 3). In marked contrast, IRAK1-deficient hearts are protected against LPS-induced contractile dysfunction. Hearts from LPS-treated IRAK1-deficient animals showed no significant decreases in maximal developed LVP, \( +dP/dt_{max} \), or \(-dP/dt_{max}\) in response to increasing coronary flow rates (Fig. 2). Similarly, hearts from IRAK1-deficient mice challenged with LPS were also resistant to the myocardial depressant effects of LPS, as determined by their systolic and diastolic responses to increasing calcium concentrations (Fig. 3). Thus IRAK1 inactivation blocks acute LPS-triggered cardiac contractile failure, implicating this kinase as a transducer of the acute myocardial depressant effects of endotoxin.

IRAK1 inactivation prolongs survival in mice that overexpress TNF-\(\alpha\) in the heart. Although IRAK1 is crucial to acute LPS-induced myocardial depression, its role in conditions characterized by chronically impaired contractility is unknown. To determine whether IRAK1 contributes to chronic contractile dysfunction as well, IRAK1 was inactivated in transgenic mice with a lethal form of heart failure. IRAK1-deficient mice were crossed with transgenic mice that overexpress TNF-\(\alpha\) in the heart. These TNF-\(\alpha\) transgenic animals

Fig. 2. IRAK1 mediates LPS-induced myocardial contractile responses to increasing coronary flow rates. Systolic and diastolic contractile function of hearts from saline- and LPS-treated (1 mg/kg ip) WT and IRAK1-deficient mice were determined using a modified Langendorff-isolated heart perfusion preparations. Maximal developed left ventricular pressure (LVP; A), rate of pressure generation (\(+dP/dt_{max}\); B), and rate of relaxation (\(-dP/dt_{max}\); C) responses of hearts to increased coronary flow were determined as described. The responses of untreated WT (n = 5) and KO (n = 7) mice, as well as saline- and LPS-treated WT (n = 9) or KO (n = 10) mice, were compared using ANOVA, followed by corrected t-tests after detection of a significant interaction between different factors (see MATERIALS AND METHODS). Data shown represent means \(\pm SE\). *\(P < 0.001\), †\(P < 0.05\).

Fig. 3. A: IRAK1 mediates LPS-induced myocardial contractile responses to increasing extracellular Ca\(^{2+}\) concentrations. B and C: responses of isolated perfused WT and KO hearts from saline- and LPS-treated animals to increasing perfusate Ca\(^{2+}\) concentrations were compared as described in Fig. 2. Data shown represent means \(\pm SE\). *\(P < 0.001\), †\(P < 0.01\).
develop myocarditis, dilated cardiomyopathy, heart failure, and experience 100% early lethality (4). Survival of the transgenic animals and the TNF-α transgenic/IRAK1 KO double mutants was compared. Figure 4A depicts the survival curves of the two strains of animals. Elimination of IRAK1 function markedly enhanced the 150-day survival of TNF-α transgenic mice. All of the transgenic animals with functional IRAK1 succumbed during the test period, whereas 70% of those lacking the kinase were still alive after 150 days. Therefore, IRAK1 inactivation protects mice against this form of lethal heart failure.

Because heart failure in the transgenic mice results from cardiac overexpression of the proinflammatory cytokine TNF-α, and IRAK1 also mediates injury sensing and inflammation at several different steps in the innate immune response, it is possible that decreased mortality was due to a diminished inflammatory response in transgenic/KO hearts. On average, hearts from 75-day-old WT mice and IRAK1 KO mice appeared normal in size and consistency. In contrast, hearts from the TNF-α overexpressors were markedly dilated, thin walled, and pale. Most hearts from double mutants (TNF-α transgenic/IRAK1 KO) appeared normal, but occasionally (<10%) a transgenic/KO animal would have a dilated heart similar to the transgenic animals. These rare animals were also clinically ill. Hearts from WT (n = 4), TNF-α transgenic (n = 7), IRAK1 KO (n = 7), and TNF-α transgenic/KO (n = 4) mice were removed for histopathological examination by a veterinary pathologist who was blinded to the animals’ genotypes. Hearts were classified as having no, mild, moderate, or severe myocarditis (Table 1). WT and IRAK1-deficient hearts lacked evident cardiac lesions (Fig. 4B). Hearts from TNF-α transgenic mice exhibited diffuse interstitial cellularity, with mononuclear inflammatory cells and fibroblasts, as well as interstitial fibrosis in both atria and ventricles, as

Fig. 4. IRAK1 deletion prevents death in lethal congestive heart failure. A: IRAK1 KO mice were interbred with animals overexpressing a tumor necrosis factor-α (TNF-α) transgene (TG) exclusively in the heart, and survival and time to death were compared using a Kaplan-Meier survival estimate. The experiment was terminated when the animals reached 150 days of age. B: histological appearance of hearts. Hearts of WT, IRAK1 KO, TNF-α TG, TNF-α TG/IRAK1 KO double mutant mice underwent blinded histological evaluation. Interstitial hypercellularity and fibrosis characterized moderate to severe myocarditis in the hearts of TNF-α transgenic mice and double mutants. Although myocarditis was generally diffuse, the foci of hypercellularity (arrows) were also present. Inset: Anitschkow-like cells in the heart of a TNF-α TG mouse.
TG, transgenic.

which also showed attenuated and delayed NF-κB-profiles that resemble those seen in macrophages (41), deficient hearts exhibit endotoxin-triggered signaling also represents a novel finding. In fact, WT and IRAK1-deficient hearts do not exhibit diminished amplitude and timing of downstream ways that result in NF-κB activation in the heart and the additional observation fails to reveal a mechanism by which IRAK1 deletion exerts this protection is unknown. Histological analysis suggests that IRAK1 deletion does not directly affect the degree of inflammation seen in transgenic hearts. Double mutant animals still synthesize NF-κB in the heart and still exhibit an inflammatory infiltrate similar to transgenic mice with IRAK1, but nonetheless live longer. Interestingly, contractile function in transgenic animals at ~50 days of life, the time when some of these animals begin to die is significantly impaired compared with double mutant animals of the same age (data not shown), but this additional observation fails to reveal a mechanism linking IRAK1 activation to worsening heart failure.

Two general possibilities could help explain this result. First, IRAK1 may be required for normal NF-κB signaling. Most studies to date have failed to uncover a role for IRAK1 in NF-κB signaling, although one report has suggested that IRAK1 potentiates IL-1 signaling to NF-κB (47). According to this view, loss of IRAK1 expression in acute LPS-mediated contractile dysfunction, IRAK1 may also be involved in chronically impaired contractility. IRAK1 inactivation enhances survival in animals with congestive heart failure due to cardiac-specific NF-κB overexpression. How IRAK1 deletion exerts this protection is unknown. Histological analysis suggests that IRAK1 deletion does not directly affect the degree of inflammation seen in transgenic hearts. Double mutant animals still synthesize NF-κB in the heart and still exhibit an inflammatory infiltrate similar to transgenic mice with IRAK1, but nonetheless live longer. Interestingly, contractile function in transgenic animals at ~50 days of life, the time when some of these animals begin to die is significantly impaired compared with double mutant animals of the same age (data not shown), but this additional observation fails to reveal a mechanism linking IRAK1 activation to worsening heart failure.

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The studies reported here show for the first time that LPS activates IRAK1 in the heart. Moreover, hearts lacking IRAK1 exhibit impaired activation of the pathways that result in NF-κB and JNK activity, displaying diminished amplitude and timing of downstream signaling required for NF-κB production in other cells (40). These findings are consistent with previously published reports. Girou and colleagues (11) first demonstrated LPS-driven cardiac TNF-α, whereas Frantz and colleagues (10) have implicated TLR4 in this cardiac-specific TNF-α response. Activation of cardiac NF-κB by LPS has also been reported previously (3, 8, 14, 36) with the observation that NF-κB inhibition protects against LPS-induced myocardial contractile dysfunction (8, 14, 36). The present study demonstrates a requirement for the Toll/IL-1 signaling intermediate IRAK1 in the cardiac signaling response to LPS. Moreover, LPS-induced JNK catalytic activation in the heart and the effect of IRAK1 inactivation on cardiac JNK signaling also represents a novel finding. In fact, WT and IRAK1-deficient hearts exhibit endotoxin-triggered signaling profiles that resemble those seen in macrophages (41), which also showed attenuated and delayed NF-κB and MAPK responses. The macrophage studies, however, were conducted in vitro, so there may be important differences in signal transduction between these two tissues. Thus the myocardium possesses an LPS-sensing apparatus, and IRAK1 regulates the cardiac signaling response to endotoxin.

The results from these studies also implicate IRAK1 in acute LPS-induced contractile dysfunction. WT LPS-treated hearts exhibited predictable and reproducible impaired contractility compared with sham-treated hearts, whereas the response of LPS-challenged IRAK1-deficient hearts was no different from sham-treated hearts lacking IRAK1. Thus IRAK1 function is required for the development of LPS-induced contractile depression.

These findings reinforce the importance of Toll/IL-1 signaling in acute myocardial contractile dysfunction by demonstrating its requirement in a second model of cardiac depression. Recent work in our laboratory (43) has demonstrated that IRAK1 transduces a signal that leads to myocardial dysfunction after large body surface area burns (35–40% total body surface area). Additional studies indicated that TLR4 was a principal sensor for burn-induced myocardial dysfunction because mice harboring inactivating TLR4 mutations failed to develop cardiac depression after injury (45). Furthermore, contractile dysfunction after burn injury could be prevented by administrations of the LPS inhibitor recombinant bactericidal/permeability-increasing protein (rBPI21) (45). Together, the results from the burn studies suggested that myocardial depression might be due to LPS translocated from the gut of injured animals, although other possible mechanisms could not be excluded. Regardless of whether LPS is responsible for myocardial depression in burn injury, the findings presented in the current experiments document a clear role for IRAK1 in contractile dysfunction after acute exogenous endotoxin administration.

In addition to its effect on acute LPS-mediated contractile dysfunction, IRAK1 may also be involved in chronically impaired contractility. IRAK1 inactivation enhances survival in animals with congestive heart failure due to cardiac-specific TNF-α overexpression. How IRAK1 deletion exerts this protection is unknown. Histological analysis suggests that IRAK1 deletion does not directly affect the degree of inflammation seen in transgenic hearts. Double mutant animals still synthesize TNF-α in the heart and still exhibit an inflammatory infiltrate similar to transgenic mice with IRAK1, but nonetheless live longer. Interestingly, contractile function in transgenic animals at ~50 days of life, the time when some of these animals begin to die is significantly impaired compared with double mutant animals of the same age (data not shown), but this additional observation fails to reveal a mechanism linking IRAK1 activation to worsening heart failure.
proinflammatory cytokine concentrations (18, 27), which may be both markers for worsening cardiac function and stimuli contributing to further deterioration in contractility. Normally, the presence of endotoxin would be expected to worsen contractile function, if the host is insensitive to this compound; however, as occurs in the double mutant mice, myocardial function might be spared. Therefore, a loss of IRAK1 function through genetic deletion may abrogate three major proximal signals that trigger cardiac dysfunction. Direct myocardial depressant actions of LPS and IL-1 are blocked, and LPS-induced TNF-α production by noncardiac cells is also impaired, such that the heart is exposed to less circulating TNF-α. Loss or diminution of these three negative inotropic inputs may slow the deterioration of contractile function in double mutant mice and thereby prolong survival. Determining the precise role for IRAK1 in lethality is this model of heart failure; however, it will require further study.

Although they identify IRAK1 as an important participant in LPS-induced myocardial dysfunction, these studies cannot pinpoint the site of IRAK1 action in this response. Myocardial protection is correlated with altered myocardial signaling responses to LPS, but it is impossible to discern whether this protection stems from disrupted signaling in the heart or some other tissue(s). LPS responsiveness is presumably disrupted in all IRAK1-deficient tissues. Thus these studies cannot determine whether the myocardial protection seen in KO mice is due to the lack of IRAK1 in the heart of its absence from some other cell type. How, then, does LPS cause myocyte contractile dysfunction? Two models, both testable, can be envisioned. In the first, LPS sensing and impaired contractility both occur in the cardiac myocyte (a cell-autonomous model). Conversely, nonmyocyte cell types either in the heart itself or elsewhere (e.g., fibroblasts, macrophages, monocytes, endocardial or endothelial cells, or hepatocytes) could sense LPS and produce secondary signals (e.g., cytokines such as TNF-α or IL-1 or other molecules) that directly depress myocardial contractility (a cell nonautonomous model). Furthermore, the models are not mutually exclusive.

Regardless of the site of the IRAK1 action, the data presented here suggest that a signaling pathway considered part of the innate immune system can regulate a nonimmune physiological response. IRAK1 inactivation blocks the usual negative effect of LPS, a bacterial product with well-described immunostimulatory properties, on cardiac contractility. Most studies of Toll/IL-1 signaling have focused on the immune consequences of pathway stimulation. This report, however, represents one of the earliest attempts to link pathway activation to physiological alterations outside the immune system. These findings have two important implications. First, demonstration that IRAK1 regulates LPS-induced contractile depression highlights the interrelatedness of different systems in integrated biological responses to infection. Second, it may force reconsideration of what properly constitutes innate immunity, usually viewed as a form of host defense mediated by bone marrow-derived cells and specialized molecules to aid in antibacterial responses, thrombosis, and inflammation.

Why, for example, does the heart, rarely a direct target of infection, have a functional Toll/IL-1 pathway? Could Toll/IL-1 signaling enact a broader injury-sensing role not limited to the detection of infection, with physiological consequences extending beyond the immune system? Recent reports seem to support this possibility. Several groups have implicated endogenous molecules, such as highly conserved heat shock proteins (28), the breakdown products of the extracellular matrix (42), and products released from necrotic cells (19) as activators of Toll/IL-1 signaling. It is therefore possible that certain Toll-like receptors on neighboring viable heart cells actually sense direct cardiac injury resulting from ischemia or trauma, when necrotic myocytes release their contents? Subsequent pathway activation could trigger responses in uninjured cells that might protect them from injury, lead to the characteristic postinjury inflammatory infiltrate, or precipitate postinjury dysfunction, to name a few possible responses. Thus the Toll/IL-1 pathway may represent a ubiquitous, generic injury sensor that serves to alert the host to damage to one of its parts and initiate the containment and healing responses.

Finally, these studies raise the possibility that IRAK1 or other members of the Toll/IL-1 signaling pathway may be targets for the therapy of cardiac contractile dysfunction. Pinpointing how IRAK1 exacerbates contractile dysfunction and limits survival represent critical first steps in developing such a strategy. The protection that genetic inactivation of IRAK1 affords mice with either acute or chronic contractile dysfunction, however, provides a theoretical basis for the development of new therapies for congestive heart failure. It may someday be possible, for example, to prolong survival in patients with end-stage heart failure through pharmacological IRAK1 inhibition.

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