Toll-like receptor 2 modulates left ventricular function following ischemia-reperfusion injury

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Am J Physiol Heart Circ Physiol 292: H503–H509, 2007. First published September 15, 2006; doi:10.1152/ajpheart.00642.2006.—Production of proinflammatory cytokines contributes to cardiac dysfunction during ischemia-reperfusion. The principal mechanism responsible for the induction of this innate stress response during periods of myocardial ischemia-reperfusion remains unknown. Toll-like receptor 2 (TLR2) is a highly conserved pattern recognition receptor that has been implicated in the innate immune response to a variety of pathogens. However, TLR2 may also mediate inflammation in response to noninfectious injury. We therefore hypothesized that TLR2 is essential for modulating myocardial inflammation and left ventricular (LV) function during ischemia-reperfusion injury. Susceptibility to myocardial ischemia-reperfusion injury following ischemia-reperfusion was determined in Langendorff-perfused hearts isolated from wild-type mice and mice deficient in TLR2 (TLR2D) and Toll interleukin receptor domain-containing adaptor protein. After ischemia-reperfusion, contractile performance was significantly impaired in hearts from wild-type mice as demonstrated by a lower recovery of LV developed pressure relative to TLR2D hearts. Creatinine kinase levels were similar in both groups after reperfusion. Contractile dysfunction in wild-type hearts was associated with elevated cardiac levels of TNF and IL-1β. Ischemia-reperfusion-induced LV dysfunction was reversed by treatment with the recombinant TNF blocking protein etanercept. These studies show for the first time that TLR2 signaling importantly contributes to the LV dysfunction that occurs following ischemia-reperfusion. Thus disruption of TLR2-mediated signaling may be helpful to induce immediate or delayed myocardial protection from ischemia-reperfusion injury.

inflammation; innate immunity; myocardial function

Reperfusion of blood flow to ischemic myocardium is in and of itself associated with a distinct form of cardiac injury that is directly attributable to the toxic effects of reactive oxygen intermediates that are generated once the heart is reperfused (6, 7, 25). Recently, it has been suggested that ischemia-reperfusion leads to increases in the expression of a portfolio of inflammatory mediators that are related to the so-called innate immune system, including TNF, IL-1β, IL-6, and nitric oxide (9, 14, 27). This robust inflammatory response provokes a number of deleterious effects in the heart with the most notable being left ventricular (LV) dysfunction (6, 8, 9). However, an incomplete understanding of the primary mechanisms through which the heart initiates this inflammatory cascade has hampered progress in the treatment of ischemia-reperfusion-induced LV dysfunction.

Recent studies have shown that the heart possesses a functionally intact innate immune system that becomes activated nonspecifically in response to all forms of acute myocardial injury, especially during ischemia-reperfusion (22, 23). Cardiac myocytes express at least four classical receptors that belong to the innate immune system (so-called pattern recognition receptors), including CD14, and Toll-like receptors 2, 4, and 6 (TLR2, TLR4, and TLR6, respectively) (12, 13, 18, 19). TLRs are highly conserved pattern recognition receptors that have been implicated in the innate immune response to a variety of pathogens (1, 26). Recently, it has become clear that TLRs also recognize endogenous host material that is released during cellular injury or death (5). All TLRs (except for TLR3) interact with an adaptor protein termed myeloid differentiation factor 88 (MyD88) via their Toll interleukin receptor domains. When stimulated, MyD88 recruits IL-1 receptor-associated kinase to the receptor complex. IL-1 receptor-associated kinase is then activated by phosphorylation and associates with tumor necrosis receptor-associated factor 6, leading to NF-κB activation (2). Although the adaptor molecule Toll interleukin receptor domain-containing adapter protein (TIRAP) was initially thought to contribute to MyD88-independent signaling, studies have shown that TIRAP is required for TLR2- and TLR4-mediated activation of NF-κB (15, 40).

Several lines of evidence support the view that TLR2 has a broad role as a pattern recognition receptor for a variety of microbes and microbial structures. These include lipoproteins from pathogens, such as mycobacteria and staphylococcal peptidoglycan and lipoteichoic acid (11, 37, 39). We have previously shown that cardiac expression of TLR2 is essential for LV dysfunction and myocardial expression of TNF, IL-1β, and nitric oxide following challenge with Staphylococcus aureus (19). However, TLR2 may also mediate innate stress responses following noninfectious injury (17). Indeed, a recent study in cardiac myocytes showed that hydrogen peroxide-induced oxidative stress was sufficient to increase signaling through TLR2 and that this signaling could be prevented by an anti-TLR2 antibody (12). Moreover, Leemans et al. (21) re-
ported that renal-associated TLR2 was an important initiator of inflammatory responses leading to renal injury and dysfunction following ischemia-reperfusion. Taken together, these observations suggest that activation of TLR2 signaling during cardiac ischemic injury may be an important link between the innate inflammatory response in the heart and the LV dysfunction associated with this condition. Accordingly, in this study, we determined whether TLR2 played a role in ischemia-reperfusion-induced cardiac inflammation and LV dysfunction.

**METHODS**

**Mice.** The mutant mice (129SV × C57BL/6) deficient in TLR2 (TLR2D) or TIRAP (TIRAP-D; provided by Dr. Ruslan Medzhitov, Yale University School of Medicine) expression were generated by gene targeting as described previously (15, 36). Homozygous TLR2(−/−) and TIRAP(−/−) mice were generated by intercrossing heterozygous (TLR2(+/−) and TIRAP(+/−)) mice. Wild-type littermates served as the appropriate controls. Male mice used in this study were maintained in specific pathogen-free conditions and were fed pellet food and water ad libitum. All studies were performed with the approval of the Institutional Animal Care and Use Committee at Baylor College of Medicine. These investigations conformed to the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health.

**Isolated heart perfusion studies.** Hearts from wild-type, TLR2D, and TIRAP-D mice were isolated and perfused in the Langendorff mode as previously described (19). Briefly, mice were injected intraperitoneally with heparin (10,000 U/kg; Sigma, St. Louis, MO) and anesthetized with Avertin (16 mg/kg body wt of a 2.5% solution). The thorax was rapidly opened and the heart excised and arrested in ice-cold saline. A short perfusion cannula was inserted into the aortic root for retrograde perfusion. Isolated hearts were perfused at a constant pressure of 80 mmHg with modified Krebs-Henseleit buffer containing (in mmol) 118 NaCl, 24 NaHCO3, 4.7 KCl, 1.2 KH2PO4, 1.2 MgSO4·7H2O, 2.2 CaCl2, 10 glucose, and 2 pyruvate (37°C), equilibrated with 95% O2-5% CO2. The perfusate was gassed with 95% O2-5% CO2 to yield a pH of 7.4. The perfusate was warmed to 37°C, filtered through a 0.22-μm filter, and kept constant pressure of 80 mmHg with modified Krebs-Henseleit buffer gassed with 95% O2-5% CO2. A handmade balloon connected to a polyethylene tube was inserted into LV through the mitral valve via an incision in the left atrium and was connected to a pressure transducer (ML844, AD Instruments, Colorado Springs, CO). The balloon was inflated with water to adjust LV end-diastolic pressure (LVEDP) at 7–10 mmHg.

After a 30-min stabilization period, hearts from wild-type TLR2D and TIRAP-D mice were subjected to 30 min of zero-flow ischemia (t = −30 min) followed by reperfusion (t = 0) for 30 to 60 min (Fig. 1). All hearts were paced at 420 beats/min with pacing electrodes placed on the right atrium. Pacing was interrupted during ischemia and resumed 3 min after the start of reperfusion. Functional data were recorded at 1 kHz on a data acquisition system (PowerLab, ADInstruments). LV developed pressure (LVDP) was calculated as the difference between peak-systolic pressure and LVEDP. At the end of each experiment, hearts were frozen in liquid nitrogen for subsequent cytokine analysis.

**Creatinine kinase assay.** Coronary effluent was collected during the first 30 min after ischemia. Creatine kinase (CK) activity was measured with a commercially available CK assay kit (Diagnostic Chemical, Charlottetown, PE, Canada) according to the manufacturer’s recommendations. CK activity was normalized for frozen-dry heart weight. Data are expressed as units per gram of cardiac tissue.

**TNF and IL-1β protein measurements.** Hearts were taken following the ischemia-reperfusion protocol, and homogenates were prepared as previously described (19). Commercially available ELISA kits (R&D Systems, Minneapolis, MN) were used for measuring myocardial TNF and IL-1β protein levels. Data are expressed as picogram per milligram of protein.

**TFN neutralization.** In separate experiments, hearts were perfused during the period before ischemia with etanercept (30 μg/ml), a recombinant fusion protein that binds TNF and functionally inactivates it (24). This concentration of etanercept has been shown to neutralize the negative inotropic effects of TNF following ex vivo ischemia-reperfusion (10). Hearts from wild-type and TLR2D mice were then subjected to ischemia-reperfusion, and LV functional recovery was measured as described in Isolated heart perfusion studies.

**Immunohistochemical staining.** To localize the cellular source of high-mobility group box 1 (HMGB1) expression, we performed immunohistochemistry studies using a rabbit anti-HMGB1 antibody (1:1,000; BD Biosciences, San Jose, CA). Paraffin-embedded cardiac sections were used for immunostaining using an immunoenzymatic staining kit (DAKO EnVision+ Systems, Peroxidase, Dako; Carpintera, CA) as recommended by the manufacturer. Counterstaining was performed with hematoxylin, and each immunostained slide was evaluated by light microscopy.

**Isolation of cytoplasmic proteins.** HMGB1 released by injured or necrotic cells has been shown to act as a signaling molecule, inducing local inflammatory responses (34). Thus, extracellular HMGB1 can be regarded as both a signal of tissue injury and a mediator of inflammation. Germaine to this discussion is the recent in vitro observation that TLR2 and TLR4 act as receptors for HMGB1 (31, 32). HMGB1 protein expression was assessed in the hearts of wild-type and TLR2D mice after ischemia-reperfusion. Hearts were homogenized in 2 ml of ice-cold extraction buffer as previously described (4). The protein concentration was determined by using the bicinchoninic assay with bovine serum albumin as a standard (Pierce, Life Science; Rockford, IL). Protein was separated on 12% SDS-polyacrylamide gel under denaturing conditions and was electroblotted onto a nitrocellulose membrane (Bio-Rad; Hercules, CA). The membrane was immunoblotted with rabbit anti-HMGB1 antibody (BD Biosciences). The primary antibody was horseradish peroxidase-conjugated goat anti-rabbit polyclonal antibody (1:1,000; Santa Cruz Biotechnology, Santa Cruz, CA). HMGB1 expression was detected with the ECL-Plus Western blotting detection kit (Amersham).

**Murine cardiac myocyte isolation.** Isolated ventricular myocytes were prepared from wild-type and TLR2D mice with minor modifications to the method described by Rockman et al. (33). The animals were anesthetized with a mixture of ketamine (100 μg/ml) and xylazine (20 μg/ml). Hearts were excised and perfused at 2 ml/min in a Langendorff apparatus, first with heart media-3 composed of (in mM) 20 glucose, 4 NaHCO3, 12 HEPES, 30 tauroine, 2 carnitine, 2 creatine, and 10 2,3-butanedione monoximine (pH 7.4) at 37°C for 5
Table 1. Baseline physiological characteristics of wildtype and TLR2D hearts

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Wildtype</th>
<th>TLR2D</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>n</td>
<td>9</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Age, wk</td>
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<td>19.2±0.7</td>
<td>0.29</td>
</tr>
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<td>Systolic LVP, mmHg</td>
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<td>88.3±3.4</td>
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<tr>
<td>LVEDP, mmHg</td>
<td>8.7±0.2</td>
<td>8.7±0.4</td>
<td>0.93</td>
</tr>
<tr>
<td>LVDP, mmHg</td>
<td>83.2±5.7</td>
<td>79.5±3.5</td>
<td>0.59</td>
</tr>
<tr>
<td>Flow rate, ml/min</td>
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<td>2.1±0.1</td>
<td>0.93</td>
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</tbody>
</table>

Values are means ± SE; n, number of mice. TLR2D, mice deficient in Toll-like receptor 2; LVP, left ventricular (LV) pressure; LVEDP, LV end-diastolic pressure; LVDP, LV developed pressure.

TLR2 deficiency improves postischemic functional recovery of isolated hearts. A control set of hearts from wild-type and TLR2D mice (n = 9) was perfused for 90 min at a constant pressure of 80 mmHg, without being subjected to ischemia. We compared systolic LV pressure, LVEDP, LVDP, and flow between the two groups. No differences were noted in the baseline physiological characteristics between wild-type and TLR2D mice (Table 1).

Hearts from wild-type and TLR2D mice were subsequently subjected to ischemia-reperfusion injury. During global zero-flow ischemia, LVEDP increased in both wild-type and TLR2D hearts. No differences were noted in LVEDP during peak contracture, the end of ischemia, or after reperfusion (data not shown). However, the recovery of postischemic contractile performance (%LVDP) was significantly greater (P ≤ 0.05) in the hearts of TLR2D mice (Fig. 2A). At 60 min after reperfusion, hearts from wild-type mice recovered 25.2 ± 1.9% of baseline LVDP, whereas hearts from TLR2D mice recovered to 33.9 ± 3.2%. These data suggest that TLR2 is involved in mediating myocardial susceptibility to ischemia-reperfusion injury.

CK release after reperfusion in wild-type and TLR2D mice. To determine whether decreased ischemia-reperfusion injury in TLR2D hearts was associated with differences in cardiac injury, CK release was measured in the perfusates during reperfusion of hearts from both wild-type and TLR2D mice. Total CK release from the heart was negligible in both groups before the induction of ischemia (data not shown). The levels of CK released were markedly elevated after reperfusion in both wild-type and TLR2D mice (Fig. 2A, inset); however, there was no significant difference between wild-type and TLR2D mice. Thus zero-flow ischemia resulted in similar myocyte injury in both groups. These data suggested that the measured difference in recovery of %LVDP between hearts from wild-type and TLR2D mice could not be explained simply by differences in the degree of ischemia-induced myocyte injury.

TIRAP deficiency decreases susceptibility to ischemia-reperfusion-induced LV dysfunction. Given the specificity of TIRAP for TLR2 signaling in several isolated cell types, we next investigated the effect of TIRAP deficiency on postischemic LV functional recovery (40). Figure 2B shows postischemic LV functional recovery in hearts from wild-type and TIRAP-D mice after ischemia-reperfusion. Ischemia-reperfusion resulted in significantly more LV dysfunction in wild-type hearts, as indicated by a lower recovery of LVDP relative to that in TIRAP-D hearts; that is, at 60 min after reperfusion, hearts from wild-type mice recovered 26.84 ± 1.5% of baseline LVDP, whereas hearts from TIRAP-D mice

![Fig. 2. Decreased susceptibility to myocardial IR injury in mice deficient in Toll-like receptor 2 (TLR2D) and Toll interleukin receptor domain-containing adaptor protein (TIRAP-D) mice. A: percent recovery of left ventricular developed pressure (%LVDP) in hearts isolated from wild-type (WT; n = 9) and TLR2D (n = 9) mice during 30 min of global zero-flow ischemia and 60 min of reperfusion. Inset: depicts the creatine kinase levels in WT (n = 7) and TLR2D (n = 7) mice after myocardial IR injury. B: %LVDP in hearts isolated from WT (n = 8) and TIRAP-D (n = 8) mice during 30 min of global zero-flow ischemia and 60 min of reperfusion. *P ≤ 0.05 vs. WT hearts.](http://ajpheart.physiology.org/Downloadedfrom/10.22033/9/11005)
recovered to 41.8 ± 1.5% \( (P \leq 0.05) \). These data support a role for TLR2-mediated TIRAP signaling in the pathogenesis of ischemia-reperfusion-induced LV dysfunction.

**Cytokine production in the heart after ischemia-reperfusion injury.** Previous studies have shown that TNF release is one of the earliest deleterious events in response to various forms of cardiac injury \( (16, 27) \). Given that TIRAP signaling has been shown to activate proinflammatory cytokines, we sought to determine whether the improvement of posts ischemic functional recovery in hearts from TLR2D mice was mediated through changes in cardiac TNF and IL-1β. Tissue homogenates from nonischemic (control) and ischemic hearts were assayed by specific TNF and IL-1β immunoassays. Figure 3, A and B, shows TNF and IL-1β protein production in the heart at 30 and 60 min after reperfusion. Consistent with the role of TNF in myocardial ischemia-reperfusion, myocardial TNF protein was significantly increased \( (P \leq 0.05) \) in hearts of wild-type mice at 30 min after reperfusion compared with hearts from TLR2D mice. No differences in TNF levels were measured between the two groups after 60 min of reperfusion. Hearts from wild-type mice also showed a trend \( (P = 0.08) \) toward higher myocardial IL-1β protein levels at 30 and 60 min of reperfusion compared with hearts from TLR2D mice. These data suggested that blunted cytokine production contributed, at least in part, to the increased posts ischemic functional recovery measured in hearts from TLR2D mice.

**Etanercept improves posts ischemic functional recovery after ischemia-reperfusion.** To determine whether TNF production played a role in the onset of LV dysfunction in our model, hearts isolated from wild-type and TLR2D mice were treated with etanercept before the onset of ischemia, as well as during resuscitation. Etanercept administration resulted in improvement of posts ischemic LV functional recovery after reperfusion in both wild-type and TLR2D hearts (Fig. 4) compared with untreated mice (Fig. 2A). As shown, there were no differences in %LVDP after etanercept administration.

**HMGB1 expression is upregulated in the heart after ischemia-reperfusion injury.** To determine whether HMGB1 was upregulated in the heart after ischemia-reperfusion injury, the cellular localization of HMGB1 in the heart was assessed by immunostaining normal perfused hearts (80 mmHg; sham-operated hearts) and hearts that underwent ischemia-reperfusion injury. Expression of HMGB1 was noted predominantly in the nucleus of normal perfused wild-type and TLR2D hearts (Fig. 5A). Consistent with reports in liver ischemic injury \( (38) \), after ischemia-reperfusion HMGB1 expression was enhanced in cytoplasm of cardiomyocytes from both wild-type and TLR2D hearts (Fig. 5A). Western blot analysis also revealed increased HMGB1 protein expression in the cytoplasmic extracts from both wild-type (2.2-fold) and TLR2D hearts (2.2-fold; Fig. 5B). Although HMGB1 expression was increased in the heart, we were unable to detect increased HMGB1 levels in the perfusates of hearts that underwent ischemia-reperfusion injury (Fig. 5C).

To determine whether low, undetectable concentrations of HMGB1 might have contributed to the TLR2-mediated LV dysfunction, we asked whether HMGB1 was sufficient to provoke cardiac myocyte dysfunction by exposing isolated murine cardiac myocytes from wild-type or TLR2D mice when they were treated with buffer. Addition of rhHMGB1 \( (1 \mu g/ml) \) induced a significant depression of myocyte contraction shortening in cells isolated from both wild-type and TLR2D mice. Thus, although HMGB1 was sufficient to provoke myocyte dysfunction, these data suggest that TLR2 is not the cognate receptor for HMGB1 in cardiomyocytes.
DISCUSSION

In this report, we demonstrate the novel finding that TLR2-TIRAP signaling contributes to the development of ischemia-reperfusion-induced LV dysfunction in the adult heart. Hearts from wild-type mice subjected to ischemia-reperfusion injury exhibited reproducible impaired LV function when compared with normal perfused (80 mmHg) hearts. More importantly, the recovery of postischemic contractile performance (%LVDP) was significantly greater ($P \leq 0.05$) in hearts from mice lacking TLR2. Given that these studies were performed in isolated perfused hearts, our results suggest that the LV dysfunction in wild-type mice was due to TLR2-mediated signaling in the heart. We provide further support by showing that the recovery of postischemic contractile performance (%LVDP) was also significantly greater ($P \leq 0.05$) in hearts from mice deficient in TIRAP, an adaptor molecule that is essential for TLR2 signaling. Moreover, when TLR4-deficient mice and their appropriate control mice were subjected to ischemia-reperfusion injury, the absence of TLR4 had no significant effect on recovery of postischemic contractile performance, suggesting that TLR4 does not play a major role in mediating LV dysfunction following ischemia-reperfusion injury (data not shown). Although the mechanisms for the difference in LV function are unknown, two lines of evidence suggest that blunted expression of proinflammatory mediators may be responsible, at least in part, for the preserved LV function in hearts from TLR2D mice subjected to ischemia-reperfusion. First, both cardiac TNF and IL-1β levels were lower in hearts from TLR2D mice after ischemia-reperfusion. Second, administration of etanercept, a selective TNF antagonist, abrogated the LV dysfunction after ischemia-reperfusion injury in wild-type mice. Although etanercept also improved the postischemic LV functional recovery in hearts from TLR2D mice, this effect was less pronounced than in wild-type mice. Taken together, these data indicate that TLR2-mediated signaling contributes to the LV dysfunction following ischemia-reperfusion injury through increased expression of inflammatory mediators. Relevant to our findings, Shishido et al. (35) recently reported that TLR2 signaling also plays a role in cardiac remodeling after myocardial infarction. Survival rates were significantly higher in TLR2D mice than in wild-type mice 4 wk after myocardial infarction, and fractional shortening was preserved at 1 and 4 wk after infarct in TLR2D mice compared with wild-type mice. In addition, myocardial fibrosis in hearts of TLR2D mice was significantly less than in wild-type mice and correlated with reduced transforming growth factor-β and collagen type I mRNA expression.

In mammalian species, there are at least 10 TLRs, and each has a distinct function in innate immune recognition (26). In response to environmental “danger” signals, represented by structural motifs not normally expressed by cells, TLRs can mediate intracellular signals that lead to inflammatory gene expression. Indeed, oxidative stress in neonatal rat cardiomyocytes has been shown to activate NF-κB via TLR2, suggesting that this receptor may be capable of monitoring cellular injury caused by ischemic stress and therefore may be crucial for the initiation of a proinflammatory innate response (12). Molecules released by stressed cells (heat shock protein 60 and HMGB1) or injured tissue (fibronectin) have been shown to activate TLR2 and TLR4 (31, 32). Tsung et al. (38) demonstrated that HMGB1 is an early mediator of injury and inflammation in liver ischemia-reperfusion and have implicated

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**Fig. 5.** High-mobility group box 1 (HMGB1) expression is upregulated in hearts after I/R. Hearts from WT and TLR2D mice underwent 30 min of zero-flow global ischemia and 60 min of reperfusion. A: immunostain of HMGB1 from sections of sham-operated hearts from WT and TLR2D mice and hearts subjected to I/R ($\times 20$ magnification). Images are representative of heart sections from 3 mice/group. B: Western blot analysis for cellular HMGB1 was performed for protein lysates from sham-operated and I/R hearts from WT and TLR2D mice. Blot shown is representative of 3 independent experiments with similar results. C: perifusates from hearts of TLR2D mice undergoing I/R were subjected to Western blot analysis of HMGB1. Blot shown is representative of 3 experiments with similar results. D: effect of recombinant human HMGB1 (rhHMGB1) on myocyte shortening. Cardiac myocytes were exposed to diluent or HMGB1, and contractility was measured in cells from WT ($n = 22$ cells) and TLR2D ($n = 23$ cells) mice as described in METHODS. *$P \leq 0.05$ vs. diluent-treated cells; NS, not significant.
TLR4 as one of the receptors that is involved in the process. It was proposed that released HMGB1 from damaged or necrotic liver cells produced an early inflammatory response by activating the TLR4 pathway. In this study we show for the first time that cardiac HMGB1 expression increases early after reperfusion of the ischemic heart. Consistent with the CK release data suggesting that zero-flow ischemia resulted in similar myocyte injury in both groups, the increase in HMGB1 expression was similar in hearts from wild-type and TLR2D mice (2.2-fold increase). Moreover, our data indicate that the improved recovery of postischemic contractile performance in TLR2D mice is not likely to be due to a diminished ability to respond to HMGB1; that is, rhHMGB1 was able to induce similar degrees of contractile dysfunction in isolated ventricular myocytes from wild-type and TLR2D mice. Thus, although HMGB1 has the potential to adversely affect cardiac myocyte contractility, this effect does not appear to be mediated via TLR2. Although at the present time it is not possible to completely discern the endogenous ligand or ligands released during ischemic injury to the heart, the data presented strongly suggest that TLR2-mediated signaling can directly enhance the expression of genes that play a role in inflammatory responses through activation of the TRIF pathway.

The findings presented herein are consistent with a growing body of literature that suggests the heart possesses an intrinsic or “innate stress response” system that is activated following tissue injury, as we and others have suggested (20). The results of our study are intriguing insofar as they suggest that classic pattern recognition receptors that are expressed by the heart, such as TLR2, -3, -4, and -6, are capable of sensing expression of genes that play a role in inflammatory responses following tissue injury, as we and others have suggested (20). The results of our study are intriguing insofar as they suggest that TLR2-mediated signaling can directly enhance the expression of genes that play a role in inflammatory responses following tissue injury. The data presented strongly suggest that TLR2-mediated signaling can directly enhance the expression of genes that play a role in inflammatory responses following tissue injury, as we and others have suggested (20).

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REFERENCES


