TLR2 ligands attenuate cardiac dysfunction in polymicrobial sepsis via a phosphoinositide 3-kinase-dependent mechanism

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CARDIOVASCULAR DYSFUNCTION is a major consequence of septic shock and contributes to the high morbidity and mortality of sepsis (19). Current wisdom implies that innate immune and inflammatory responses are involved in the pathophysiology of sepsis, shock, and multiple organ failure that ultimately leads to the death of the host (26). Despite extensive investigation, the mechanisms by which innate immune and inflammatory responses are involved in myocardial dysfunction during septic shock have remained elusive.

Toll receptors are an ancient and evolutionarily conserved receptor family that regulates innate immunity, inflammation, and antimicrobial host defense (22). More than 10 Toll-like receptors (TLRs) have been identified in mammals (6, 8) since a human homolog of Toll, designated as TLR, was discovered in 1997 (22). TLRs recognize cell wall products from various pathogens and transduce an activation signal into the cell (6, 39). For example, TLRs (TLR1, TLR2, TLR4, TLR5, and TLR6), which are expressed on the cell surface, are involved in the recognition of structures unique to bacteria or fungi, whereas TLRs that are localized in intracellular compartments (TLR3, TLR7, TLR8, and TLR9) will recognize viral or bacterial nucleic acids (1). TLRs have been implicated in cardiac dysfunction in several important disease states, including ischemia-reperfusion injury (9), congestive heart failure (10), and septic shock (34). TLR-mediated signaling predominately activates NF-κB, which is an important transcription factor controlling the expression of inflammatory cytokine genes. We have reported that NF-κB binding activity was significantly increased in the organs of cecal ligation and puncture (CLP) mice (3, 33).

TLR4 has been reported to contribute to cardiac dysfunction in lipopolysaccharide or CLP-induced polymicrobial sepsis (25). However, the role of TLR2 in sepsis-induced cardiomyopathy remains unclear. Using an in vitro Langendorff perfusion apparatus, Knuefmann et al. (18) reported that the addition of Staphylococcus aureus into the perfusate decreased cardiac contractility in wild-type (WT) mice but not in TLR2-deficient mice. We have previously reported that glucan phospho-activated cellular signaling through a Dectin/TLR2-mediated pathway (4), which attenuates cardiac dysfunction in CLP sepsis (15) and increases the survival outcome in polymicrobial sepsis (34), suggesting that the activation of TLR2 may play a beneficial role in CLP-induced cardiac dysfunction.

We have reported that the activation of the phosphoinositide 3-kinase (PI3K)/Akt pathway correlates with attenuated cardiac dysfunction (15) and improved survival outcome in CLP sepsis (35). Recent evidence suggests that there is a cross talk between TLR signaling and the PI3K/Akt pathway (12, 36). The stimulation of TLRs activates the PI3K/Akt pathway, which has been shown to prevent cardiac myocyte apoptosis and protect the myocardium from ischemia-reperfusion injury (16, 17). Our laboratory (20, 35) and others (12, 14) have reported that the PI3K/Akt signaling pathway may be an endogenous negative feedback regulator and/or compensatory mechanism that serves to limit proinflammatory and apoptotic events in response to injurious stimuli. The activation of the PI3K/Akt signaling pathway is associated with increased survival in CLP-septic mice (35) and decreased myocardial ischemic injury (20). Interestingly, the stimulation of TLR2 activated the...
PI3K/Akt signaling pathway (2). Recent evidence suggested that Mal, an adaptor protein in the TLR-mediated signaling pathway, connects TLR2 to PI3K activation (30). Therefore, it is possible that the modulation of TLR2 will activate the PI3K/Akt signaling pathway during sepsis/septic shock.

In the present study, we examined the effect of the TLR2 ligands, peptidoglycan (PGN), and Pam3CSK4 on cardiac function indexes such as the rate of change of left ventricular (LV) pressure (dP/dt), stroke work, cardiac output, and ejection fraction (EF) in CLP-induced septic mice. We observed that the administration of TLR2 ligands significantly attenuated cardiac dysfunction in CLP sepsis. The therapeutic administration of Pam3CSK4 1 h after CLP also significantly improved cardiac function in CLP mice. In addition, the TLR2 ligand significantly increased the activation of the PI3K/Akt signaling pathway. Importantly, TLR2 deficiency or PI3K inhibition abolished the cardioprotective effect of the TLR2 ligands. These data suggest that the modulation of TLR2 may be useful in preventing cardiac dysfunction and that the mechanism involves the activation of the PI3K/Akt signaling pathway.

MATERIALS AND METHODS

Experimental animals. Age- and weight-matched male C57BL/6 mice and TLR2 knockout mice (B6.129-TLR2<sup>mut−/−</sup>) were obtained from Jackson Laboratory (Indianapolis, IN). The mice were maintained in the Division of Laboratory Animal Resources at East Tennessee State University. The experiments outlined in this article conform to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, Revised 1996). All aspects of the animal care and experimental protocols were approved by the East Tennessee State University Committee on Animal Care.

CLP polymicrobial sepsis model. CLP was performed to induce sepsis in mice as previously described (15, 32, 37). Briefly, the mice were anesthetized by 5.0% isoflurane. The anesthesia was maintained by inhalation of 1.5% isoflurane driven by 100% oxygen flow and ventilated using a rodent ventilator. A midline incision was made on the anterior abdomen, and the cecum was exposed and ligated with a 4-0 suture. Two punctures were made through the cecum with an 18-gauge needle, and feces were extruded from the holes. The abdomen was then closed in two layers. Sham surgically operated mice served as the surgery control group. Immediately following surgery, a single dose of resuscitative fluid (lactated Ringer solution, 50 ml/kg body wt) was administered by subcutaneous injection (15).

Experimental design. To examine the effect of TLR2 ligands on cardiac function during sepsis, mice (n = 8/group) were treated with and without PGN (Sigma Chemical, St. Louis, MO) at 50 μg/25 g body wt by intraperitoneal injection 1 h before surgery. Sham surgically operated mice served as the sham-operated control (n = 4/group). Six hours after surgery, hemodynamic measurements were performed using the Millar conductance catheter system (Millar Instruments, Houston, TX) as described previously (15). We also examined the effect of posttreatment with the TLR2 ligand on cardiac function in CLP mice. Pam3CSK4 (50 μg/25 g body wt) was administered to the mice (6/group) 1 h after CLP. Cardiac function was measured by echocardiography before CLP and 6 h after CLP (31). The optimal dose was selected from the dose-range preliminary studies. In addition, 6 h after CLP is an optional time for the measurement of cardiac function based on our previous studies (15, 38).

To determine the role of TLR2 in cardiac function during sepsis, TLR2-deficient mice (n = 6/group) were treated with PGN (50 μg/25g body wt) by intraperitoneal injection 1 h before CLP. Cardiac function was examined by the Millar conductance catheter system (15) and echocardiography as described previously (31) before surgery and 6 h after CLP.

To evaluate the role of the PI3K/Akt signaling pathway in TLR2 ligand-induced cardioprotection, the PI3K-specific inhibitor LY-294002 (LY; 1 mg/25 g body wt) was administered to mice (n = 6/group) 15 min before PGN administration (16, 17, 35). CLP was performed as described above. Sham surgically operated mice served as sham-operated control (n = 4). The experimental groups were sham + LY, CLP + LY, sham + LY + PGN, and LY + PGN + CLP. Cardiac function was examined by echocardiography before surgery and 6 h after CLP.

Hemodynamic measurements. Mice were anesthetized by 5.0% isoflurane. The anesthesia was maintained by inhalation of 1.5% isoflurane driven by 100% oxygen flow and ventilated using a rodent ventilator. A Millar conductance catheter system (Millar Instruments) was positioned in the left ventricle via the right carotid artery for continuous registration of LV pressure-volume loops (15) using the PowerLab system (AD Instruments, Colorado Springs, CO) as described previously (15). Indexes of systolic and diastolic cardiac performance were derived from LV pressure-volume data obtained at steady state.

Echocardiography. Transthoracic two-dimensional M-mode echocardiogram and pulsed-wave Doppler spectral tracings were obtained using a Toshiba Apio 80 imaging system (Tochigi, Japan) equipped with a 12-MHz linear transducer as described previously (31). The mice were anesthetized by 5.0% isoflurane. The anesthesia was maintained by inhalation of 1.5% isoflurane driven by 100% oxygen flow and ventilated using a rodent ventilator. Body temperature was maintained at 37°C using a heating pad. M-mode tracings were used to measure LV wall thickness, LV end-systolic diameter, and LV end-diastolic diameter. Percent fractional shortening (%FS) was calculated as described previously (31).

Western blot analysis. Immunoblots were performed as described previously (15, 35). Briefly, the cellular proteins from hearts were separated by SDS-polyacrylamide gel electrophoresis and transferred onto Hybond enhanced chemiluminescence (ECL) membranes (Amersham Pharmacia, Piscataway, NJ). The ECL membranes were incubated with appropriate primary antibody [anti-phospho-Akt, anti-Akt, anti-phospho-GSK-3β (anti-Ser9), and anti-GSK-3β (Cell Signaling Technology, Beverly, MA)], followed by an incubation with peroxidase-conjugated second antibodies (Cell Signaling Technology). The membranes were analyzed by the ECL system (Amersham Pharmacia). The signals were quantified by scanning densitometry and computer-assisted image analysis.

Statistical analysis. Data are expressed as means ± SE. Comparisons of data between groups were made using one-way ANOVA, and Tukey’s procedure for multiple-range tests was performed. P < 0.05 was considered to be significant.

RESULTS

TLR2 ligands attenuated cardiac dysfunction in CLP-induced sepsis. To examine the effect of TLR2 ligands on cardiac function in CLP-induced sepsis, we treated mice with PGN (50 μg/25 g body wt) 1 h before the mice were subjected to CLP. Untreated mice were also subjected to CLP. Sham surgically operated mice served as sham control. Fluid resuscitation was immediately performed following surgical operation. Six hours after CLP, hemodynamic measurements were performed as described previously using the Millar conductance catheter system (15). As shown in Fig. 1A, CLP resulted in the suppression of cardiac function as evidenced by decreased (P < 0.05) maximum dP/dt (dP/dt<sub>max</sub>; 47.5%), stroke work (73.7%), cardiac output (78.5%), and EF (45.5%). The cardiac dysfunction was not due to CLP-reduced circulating volume because the levels of end-systolic volume in CLP mice following fluid
Fig. 1. Toll-like receptor 2 (TLR2) ligands, peptidoglycan (PGN), and Pam3CSK4 (Pam3) attenuated cardiac dysfunction in cecal ligation and puncture (CLP)-induced sepsis. 

A: PGN was administrated to mice by intraperitoneal injection 1 h before the mice were subjected to CLP. Surgically operated mice served as sham-operated control. Fluid resuscitation was immediately performed following surgical operation. Six hours after CLP, left ventricle hemodynamic parameters were measured using a microconductance pressure catheter (Millar Instruments). 

B: Pam3CSK4 was administered 1 h after CLP. Cardiac function was evaluated by echocardiography before and 6 h after CLP. There were 4–8 mice in each group. *\(P < 0.05\) compared with indicated groups. Sham, sham-operated control.

dP/dt \(_{\text{max}}\), maximum rate of change in left ventricular pressure; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; SW, stroke work; CO, cardiac output; EF, ejection fraction; PES, end-systolic pressure; PED, end-diastolic pressure; VES, end-systolic volume; VED, end-diastolic volume.
resuscitation were not reduced compared with sham-operated control. The data suggest that fluid resuscitation immediately following surgical operation maintained the circulating blood volume in CLP-induced septic mice, which is consistent with our previous report (15).

In contrast, cardiac dysfunction was significantly attenuated in PGN-treated CLP mice. PGN administration increased (P < 0.05) cardiac output by 299%, EF by 78.7%, dP/dt max by 55.3%, and stroke work by 146% compared with the levels in the CLP group.

Echocardiographic examination also showed that CLP resulted in significant cardiac dysfunction. As shown in Table 1, EF was reduced by 27.4% and fractional shortening by 35.9% compared with those before CLP. PGN administration significantly attenuated CLP-decreased EF, %FS, LV end-systolic volume, and LV end-diastolic volume compared with the levels in untreated CLP mice (Table 1).

We also examined the effect of another TLR2 ligand, Pam3CSK4, that was administered therapeutically, 1 h after CLP, on cardiac function in CLP mice. Cardiac function was examined using the Millar conductance catheter system (15) 6 h after CLP. As shown in Fig. 2A, Pam3CSK4 treatment significantly attenuated CLP-induced cardiac dysfunction. Both EF and %FS were increased by 43.5% and 58.7%, respectively, by Pam3CSK4 compared with the levels in untreated CLP mice.

TLR2 deficiency abolished TLR2 ligand-induced cardioprotection in CLP-induced sepsis. To confirm that the ligand-induced cardioprotection in CLP mice requires TLR2, we examined the effect of TLR2 deficiency on cardiac function in CLP mice. Cardiac function was examined before and 6 h after CLP. As shown in Fig. 1B, Pam3CSK4 treatment significantly attenuated CLP-decreased EF, %FS, LV end-systolic volume, and LV end-diastolic volume compared with the levels in untreated CLP mice (Table 1).

We next examined the role of TLR2 deficiency in ligand-induced cardioprotection in CLP sepsis. We first administered PGN to TLR2−/− and WT mice 1 h before CLP. Cardiac function was examined by echocardiography before and 6 h after CLP. As shown in Table 1, CLP resulted in cardiac dysfunction as evidenced by a decrease in EF and %FS in both WT and TLR2−/− mice. PGN administration significantly attenuated CLP-induced cardiac dysfunction in WT mice. However, the beneficial effect of PGN on cardiac function in CLP-induced sepsis was abolished in TLR2−/− mice. In TLR2−/− mice, CLP significantly reduced EF by 21.8% and %FS by 29.8%.

We also examined the effect of TLR2 deficiency on the beneficial effect of Pam3CSK4 therapeutic treatment on cardiac dysfunction following CLP. Pam3CSK4 was administered to WT and TLR2−/− mice 1 h after CLP. Cardiac function was evaluated before and 6 h after CLP. As shown in Fig. 2B, the beneficial effect of Pam3CSK4 therapy on cardiac dysfunction in WT mice was lost in TLR2−/− mice. When taken together, the data suggest that TLR2 ligand-induced cardiac protection is mediated through a TLR2-dependant mechanism.

**Table 1. PGN-induced cardioprotection in CLP sepsis is mediated through a TLR2- and PI3K-dependent mechanism**

<table>
<thead>
<tr>
<th>Heart Rate, beats/min</th>
<th>%EF</th>
<th>%FS</th>
<th>LVESD, mm</th>
<th>LVEDD, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT pre-CLP</td>
<td>372.4 ± 15.51</td>
<td>54.0 ± 1.46</td>
<td>27.6 ± 0.95</td>
<td>2.77 ± 0.05</td>
</tr>
<tr>
<td>WT CLP</td>
<td>359.3 ± 19.47</td>
<td>39.2 ± 0.92</td>
<td>17.7 ± 0.41</td>
<td>1.65 ± 0.11</td>
</tr>
<tr>
<td>WT pre-CLP PGN</td>
<td>380.1 ± 18.19</td>
<td>56.7 ± 2.27</td>
<td>29.5 ± 1.59</td>
<td>2.63 ± 0.08</td>
</tr>
<tr>
<td>WT CLP PGN</td>
<td>418.9 ± 12.91b</td>
<td>59.1 ± 2.51b</td>
<td>30.4 ± 1.53b</td>
<td>2.13 ± 0.19b,c</td>
</tr>
<tr>
<td>TLR2−/− pre-CLP</td>
<td>425.0 ± 9.50</td>
<td>58.6 ± 3.14</td>
<td>30.9 ± 1.72</td>
<td>2.25 ± 0.13</td>
</tr>
<tr>
<td>TLR2−/− CLP</td>
<td>514.4 ± 16.25c</td>
<td>45.8 ± 1.24c</td>
<td>21.7 ± 0.76c</td>
<td>1.93 ± 0.12</td>
</tr>
<tr>
<td>TLR2−/− pre-CLP PGN</td>
<td>443.6 ± 8.35</td>
<td>58.5 ± 0.88</td>
<td>30.2 ± 0.65</td>
<td>2.32 ± 0.12</td>
</tr>
<tr>
<td>TLR2−/− CLP PGN</td>
<td>490.8 ± 6.70a</td>
<td>47.9 ± 0.82a</td>
<td>23.0 ± 0.57a</td>
<td>2.02 ± 0.13</td>
</tr>
<tr>
<td>Pre-LY</td>
<td>370.7 ± 10.37</td>
<td>56.5 ± 2.23</td>
<td>29.1 ± 1.48</td>
<td>2.60 ± 0.09</td>
</tr>
<tr>
<td>LY-CLP</td>
<td>336.0 ± 12.42</td>
<td>36.8 ± 1.60</td>
<td>16.9 ± 0.75</td>
<td>2.00 ± 0.08</td>
</tr>
<tr>
<td>Pre-LY-PGN</td>
<td>410.9 ± 14.41</td>
<td>51.9 ± 1.90</td>
<td>26.2 ± 1.14</td>
<td>2.60 ± 0.09</td>
</tr>
<tr>
<td>LY-PG-PGN</td>
<td>371.3 ± 5.58</td>
<td>37.6 ± 1.90</td>
<td>17.3 ± 0.98</td>
<td>2.22 ± 0.12</td>
</tr>
</tbody>
</table>

Values are means ± SE. Peptidoglycan (PGN) was administered to Toll-like receptor 2 knockout (TLR2−/−) and age-matched wild-type (WT) mice 1 h after cecal ligation and puncture (CLP). Phosphoinositide 3-kinase (PI3K) inhibitor LY-294002 (LY) was also injected into WT mice 15 min before PGN administration. CLP was induced in those mice, and surgically operated mice served as sham-operated control group. Cardiac function was examined by echocardiography before and 6 h after CLP. There were 6 mice in each group. Pre-CLP, before CLP; EF, ejection fraction; FS, fractional shortening; LVESD, left ventricular end-systolic diameter; LVEDD, left ventricular end-diastolic diameter. *P < 0.05 compared with WT pre-CLP; †P < 0.05 compared with WT CLP; ‡P < 0.05 compared with TLR2−/− pre-CLP; ¶P < 0.05 compared with TLR2−/− pre-CLP PGN; ¤P < 0.05 compared with pre-PGN; 1P < 0.05 compared with PGN-CLP.
Pharmacological inhibition of PI3K abrogates PGN-induced cardioprotection in CLP sepsis. To examine whether the increased activation of the PI3K/Akt pathway is responsible for the attenuation of cardiac dysfunction in PGN-treated CLP mice, we administered the PI3K inhibitor LY to PGN-treated mice before CLP and examined cardiac function by echocardiography. As shown in Table 1, the pharmacological inhibition of PI3K with LY abrogated the beneficial effect of PGN on cardiac dysfunction in CLP mice. The EF (37.6 ± 1.90) and %FS (17.3 ± 0.98) in the PGN/LY-treated group were significantly lower compared with the PGN-treated mice that did not receive the inhibitor (59.1 ± 2.51 and 30.4 ± 1.53, respectively) after CLP. The administration of LY did not significantly affect CLP-induced cardiac dysfunction in non-PGN-treated mice.

**DISCUSSION**

A significant finding in the present study is that TLR2 ligands, either pretreatment or therapeutic administration, at-
tenuate cardiac dysfunction in CLP-induced sepsis. However, this beneficial effect was lost in TLR2^{−/−} mice, suggesting that TLR2 is required for ligand-induced cardioprotection. In addition, PI3K inhibition abolished the cardioprotection resulting from PGN administration in septic mice. Our data suggest that the administration of TLR2 ligands attenuates cardiac dysfunction in an acute model of septic shock via a TLR2- and PI3K-dependent mechanism. More significantly, the therapeutic treatment of CLP mice with Pam3CSK4, which is a synthetic TLR2 ligand, showed clinical relevance.

Clinical and experimental studies have shown that myocardial dysfunction is an early and fatal complication of septic shock (19). We have previously shown that glucan phosphate significantly increased survival in CLP mice (33) and attenuated cardiac dysfunction in CLP-induced septic mice (15). Glucan phosphate-induced cellular signaling is mediated through a Dectin/TLR2 pathway (4), suggesting that the modulation of the TLR2 signaling pathway could be beneficial during sepsis/septic shock. In the present study, we observed that TLR2 modulation with TLR2 ligands significantly attenuated cardiac dysfunction in CLP-induced sepsis/septic shock. We demonstrated that cardioprotection induced by the ligands was abolished in TLR2-deficient mice, suggesting that the presence of TLR2 is essential for the cardioprotection induced by PGN or Pam3CSK4 administration.

Our data suggest that TLR2 plays a cardioprotective role in response to CLP-induced sepsis. Recent studies have shown that the pretreatment of mice with PGN improved bacterial clearance and decreased inflammation and mortality in mice challenged with *Pseudomonas aeruginosa* (24) or *S. aureus* (23). However, Knuefermann et al. (18) reported that TLR2 signaling contributed to the loss of myocardial contractility in mouse hearts that were directly subjected to *S. aureus* challenge in vitro. These authors isolated hearts from TLR2 knock-out mice and age-matched WT mice and examined cardiac function using a Langendorff perfusion apparatus before and after the addition of heat killed *S. aureus* into the perfusate (10^8 colony-forming units/ml). WT hearts treated with *S. aureus* exhibited significant contractile dysfunction compared with control hearts. Hearts from TLR2-deficient mice were protected from *S. aureus*-induced contractile dysfunction (18). The difference between our results and the results of Knuefermann et al. (18) may reflect differences in the experimental models. Specifically, we employed the CLP model versus the ex vivo model employed by Knuefermann et al. (18). Additional studies will be required to more fully delineate the role of TLR2 in response to sepsis.

We observed that PGN administration significantly increased the levels of phosphorylated Akt in the myocardium, suggesting that the PI3K/Akt signaling pathway was activated. PI3K is a conserved family of signal transduction enzymes, which is involved in regulating cellular proliferation and survival (5). Akt is an important physiological mediator in the PI3K pathway and when activated, modulates cell cycle entry, growth, and survival (5). Recent evidence suggests that the stimulation of TLRs leads to the activation of the PI3K/Akt signaling pathway (2, 27). For example, the stimulation of TLR2 results in the recruitment of active Rac1 and PI3K to the TLR2 cytosolic domain, resulting in the activation of the PI3K/Akt pathway (2). We observed that PGN administration significantly induced TLR2 tyrosine phosphorylation and increased the association of PI3K p85 with TLR2 (T. Ha, Y. Hu, L. Liu, C. Lu, J. McMullen, T. Shioi, S. Izumo, J. Kelley, R. Kao, D. Williams, X. Gao, and C. Li, unpublished observations). When considered together, these data indicate that PGN administration could promote TLR2 association with the p85
subunit of PI3K, resulting in the activation of PI3K/Akt-dependent signaling.

At present, we do not fully understand how the activation of the PI3K/Akt signaling pathway maintains cardiac function during polymicrobial sepsis. However, it has been well documented that innate immune and inflammatory responses play a role in cardiac dysfunction during sepsis/septic shock. We demonstrated that the administration of TLR2 ligands significantly increased the activation of the PI3K/Akt signaling pathway. There is compelling evidence that the PI3K/Akt signaling pathway functions as a negative feedback regulator of TLR-mediated NF-κB activation pathways (12–14, 29, 36). Guha and Mackman (14) reported that the activation of the PI3K/Akt signaling pathway limits the proinflammatory effects of lipopolysaccharide in cultured monocytes. Our laboratory (15) has previously reported that the activation of the PI3K/Akt signaling pathway positively correlates with decreased NF-κB activation, a critical transcription factor controlling inflammatory gene expression, in the myocardium in CLP-induced septic mice. We have also shown that PI3K inhibition significantly increased systemic cytokine levels and decreased survival outcome in CLP-septic mice (35). These data suggest that PI3K/Akt may be a negative feedback mechanism that prevents excessive innate immune and/or inflammatory responses during sepsis/septic shock (12, 13, 36).

In addition to its role as a negative feedback regulator of inflammatory responses, PI3K/Akt has been reported to directly regulate cardiac myocyte contractility (7). Condorelli et al. (7) reported that transgenic mice with cardiac-specific expression of Akt showed a significant increase in cardiac contractility compared with WT mice. Rota et al. (28) found that transgenic mice with nuclear targeting of Akt in cardiac myocytes show significantly enhanced ventricular function and myocyte contractility. The increased myocyte contractility with nuclear-targeted Akt was related to influx of Ca^{2+} into its site of storage with increased loading of the sarcoplasmic reticulum. Further studies are needed to elucidate whether there is a change of Ca^{2+} influx in the myocardium during CLP-induced polymicrobial sepsis/septic shock.

In summary, TLR2 ligand prophylaxis and therapy attenuated cardiac dysfunction in CLP-induced sepsis. The mechanism involves the activation of the PI3K/Akt signaling pathway in the myocardium. To verify this mechanism, we demonstrated that the pharmacological inhibition of PI3K abolished the beneficial effect of PGN administration on cardiac function during CLP-sepsis/septic shock. The present study also demonstrated that TLR2 is essential for maintaining cardiac function in CLP-mice and that the beneficial effect of TLR2 modulation by its ligands on cardiac function is mediated through a PI3K/Akt-dependent mechanism. Future studies are needed to determine the mechanism by which the activation of the PI3K/Akt pathway protects myocardial function during sepsis.

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DISCLOSURES

There were no conflicts of interest for the authors in the present study.

REFERENCES


