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CARDIAC-SPECIFIC OVEREXPRESSION OF INSULIN-LIKE GROWTH FACTOR I (IGF-1) RESCUES LIPOPOLYSACCHARIDE-INDUCED CARDIAC DYSFUNCTION AND ACTIVATION OF STRESS SIGNALING IN MURINE CARDIOMYOCYTES

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Abstract

LPS, a component of the outer membrane of Gram-negative bacteria, plays a key role in cardiac dysfunction in sepsis. Low circulating levels of insulin-like growth factor 1 (IGF-1) are found in sepsis, although the influence of IGF-1 on septic cardiac defect is unknown. This study was designed to examine the impact of IGF-1 on LPS-induced cardiac contractile and intracellular Ca²⁺ dysfunction, activation of stress signal, and endoplasmic reticulum (ER) stress. Mechanical and intracellular Ca²⁺ properties were examined in cardiomyocytes from Fast Violet B and cardiac-specific IGF-1 overexpression mice treated with or without LPS (4 mg kg⁻¹, 6 h). Reactive oxygen species (ROS), protein carbonyl formation, and apoptosis were measured. Activation of mitogen-activated protein kinase pathways (p38, c-jun N-terminal kinase [JNK] and extracellular signal-related kinase [ERK]), ER stress, and apoptotic markers were evaluated using Western blot analysis. Our results revealed decreased peak shortening and maximal velocity of shortening/relengthening and prolonged duration of relengthening in LPS-treated Fast Violet B cardiomyocytes associated with reduced intracellular Ca²⁺ decay. Accumulation of ROS protein carbonyl and apoptosis were elevated after LPS treatment. Western blot analysis revealed activated p38 and JNK, up-regulated Bax, and the ER stress markers GRP78 and Gadd153 in LPS-treated mouse hearts without any change in ERK and Bcl-2. Total protein expression of p38, JNK, and ERK was unaffected by either LPS or IGF-1. Interestingly, these LPS-induced changes in mechanical and intracellular Ca²⁺ properties, ROS, protein carbonyl, apoptosis, stress signal activation, and ER stress markers were effectively ablated by IGF-1. In vitro LPS exposure (1 µg mL⁻¹) produced cardiomyocyte mechanical dysfunction reminiscent of the in vivo setting, which was alleviated by exogenous IGF-1 (50 nM). These data collectively suggested a beneficial of IGF-1 in the management of cardiac dysfunction under sepsis.

Keywords

IGF-1; Sepsis; Cardiomyocytes; Oxidative stress; Stress signaling

INTRODUCTION

Sepsis, the systemic response to infection, is initiated through the effects of one or more components of the invading microorganisms, including structural elements such as endotoxin, from the Gram-negative bacteria LPS (1,2). Impaired cardiac contractile function usually dominates the clinical presentation in septic patients, leading to myocardial depression characterized by reversible biventricular dilatation, decreased ejection fraction, and profound systemic vasodilation with decreased response to fluid resuscitation (3-5). Activation or up-regulation of multiple stress signaling cascades such as iNOS, oxidative stress, and mitogen-activated protein kinase have been speculated to play a pivotal role in the pathogenesis of sepsis-associated cardiac contractile dysfunction (6-9). This notion is supported by the beneficial effects of antioxidants, free radical scavengers, and peroxisome proliferator-activated receptor- α agonists against septic complications (10,11). Nonetheless, unique and efficacious clinical management for sepsis-associated myocardial dysfunction and heart failure has been somewhat lacking, which is responsible for the high cardiac mortality in septic patients.

Insulin-like growth factor 1 (IGF-1), a critical cardiac survival factor, is known to regulate protein metabolism, facilitate protein synthesis, promote cell growth, and protect against cell death. Insulin-like growth factor 1 improves myocardial contraction, hemodynamics, and energy metabolism, as well as protects the heart against apoptosis induced by ischemia or oxidative stress (12). Transgenic mice with cardiac-specific overexpression of IGF-1 display reduced cardiomyocyte apoptosis, lessened ventricular wall stress, and chamber dilatation after myocardial infarction or during aging (13-15), indicating a critical role of this essential cardiac survival factor in the maintenance of cardiac morphology and function. It has been shown that circulating IGF-1 levels are drastically reduced in patients with sepsis, which is in line with the beneficial role of IGF-1 supplementation on the overall survival rate in sepsis, possibly via a facilitated hepatic bacterial clearance and improved cellular immune response (16,17). However, little is known with regards to the impact of IGF-1 on sepsis-induced cardiac contractile dysfunction. This study was designed to examine the effect of IGF-1 on LPS-induced cardiac contractile response and activation of stress signaling. Cardiomyocyte contractile and intracellular Ca^{2+} properties, accumulation of reactive oxygen species (ROS), protein carbonyl formation, apoptosis, and mitogen-activated protein kinase (MAPK) stress signaling cascades (p38, c-jun N-terminal kinase [JNK], and extracellular signal related kinase [ERK]) were evaluated in adult wild-type Fast Violet B (FVB) and transgenic mice with cardiac-specific overexpression of IGF-1 treated with or without LPS. Because endoplasmic reticulum (ER) stress is known to be closely associated with septic shock (18), which may contribute to cardiac contractile dysfunction (19); crucial protein markers of ER stress such as GRP78 and Gadd153 were also monitored in myocardium of IGF-1 transgenic and FVB mice with or without LPS challenge. To discern the cardiac-specific versus systemic effect of the endotoxin, the effect of LPS on cardiomyocyte mechanical function was also examined in vitro in the presence or absence of exogenous IGF-1.

MATERIALS AND METHODS

Experimental animals, LPS treatment, and IGF-1 assay

All animal procedures used in this study were approved by the Animal Care and Use Committee at the University of Wyoming (Laramie, Wyo). Weight- and age- (4–5 months of age) matched

adult female FVB and IGF-1 transgenic mice were used. Generation of the IGF-1 transgenic mice was described in detail previously (20). In brief, FVB mice were used as embryo donors. Founder mice were generated by microinjection of the male pronucleus of fertilized mouse eggs with the 2.8-kb α -major histocompatibility complex/IGF-1B construct and the 4.5-kb tyrosinase minigene (used as a coat selection marker). Microinjected eggs were implanted into the oviduct of pseudopregnant female mice and carried to term. Positive founders were then bred to wild-type FVB mice. All IGF-1B-positive mice in F1 generation showed light brown color, and all IGF-1B negative littermates were white; it was assumed that the two transgenes (IGF-1B and tyrosinase) were integrated into the same chromosomal location. Further littermates were screened by visual inspection for the coat color (IGF-1, light brown; FVB, white). Polymerase chain reaction of genomic DNA was routinely performed to ensure that genetic recombination had not occurred (20). All animals were kept in our institutional animal facility with free access to standard laboratory chow and tap water. On the day of experimentation, both FVB and IGF-1 transgenic mice were injected with 4 mg kg⁻¹ Escherichia coli LPS (i.p.) dissolved in sterile saline or an equivalent volume of pathogen-free saline (for control groups). The dosage of LPS injection was chosen based on previous observation of overt myocardial dysfunction without significant mortality (21). Mice were killed for experimentation 6 h after LPS challenge. Serum IGF-1 was analyzed using an enzyme-linked immunosorbent assay in accordance with manufacturer's recommendations (R & D System Inc, Minneapolis, Minn). All samples were analyzed in duplicate.

Isolation of mouse cardiomyocytes and in vitro LPS treatment

Cardiomyocytes were enzymatically isolated as described previously (22). In brief, mice were anesthetized using ketamine and xylazine (3:5; 1.32 mg kg⁻¹). Hearts were rapidly removed and perfused with oxygenated (5% CO₂/95% O₂) Krebs-Henseleit bicarbonate (KHB) buffer containing 118 mM NaCl, 4.7 mM KCl, 1.25 mM CaCl₂, 1.2 mM MgSO₄, 1.2 mM KH₂PO₄, 25 mM NaHCO₃, 10 mM HEPES, and 11.1 mM glucose. Hearts were then perfused with a Ca²⁺-free KHB containing Liberase Blendzyme 4 (Hoffmann-La Roche Inc., Indianapolis, Ind) for 20 min. After perfusion, left ventricles were removed and minced to disperse cardiomyocytes in Ca²⁺-free KHB buffer. Extracellular Ca²⁺ was added incrementally back to 1.25 mM. Myocyte yield was approximately 70%, which was not overtly affected by either LPS or IGF-1. Only rod-shaped myocytes with clear edges were selected for mechanical and intracellular Ca²⁺ transient studies. Cells were used for functional or biochemical assessment within 6 h of isolation. To elucidate the cardiac specificity, if any, of LPS-induced cardiomyocyte mechanical response, a sublethal dose of E. coli LPS (1 μ g mL⁻¹) was administered to cardiomyocytes isolated from FVB mice maintained in Medium 199 with Earle salts containing HEPES (25 mM) and NaHCO₃ (25 mM), supplemented with albumin (2 mg mL⁻¹), l-carnitine (2 mM), creatine (5 mM), taurine (5 mM), insulin (100 nM), penicillin (100 U mL⁻¹), streptomycin (100 μ g mL⁻¹), and gentamicin (5 μ g mL⁻¹). The cells were incubated with LPS for 4 to 6 h in the presence or absence of exogenous recombinant IGF-1 (50 nM). The dose and duration of LPS and IGF-1 treatment were based on previously published reports (23,24).

Cell shortening/relengthening measurement

The mechanical properties of cardiomyocytes were assessed using a SoftEdge MyoCam system (IonOptix Corporation, Milton, Mass) (22). In brief, left ventricular cardiomyocytes were placed in a chamber mounted on the stage of an inverted microscope (IX-70; Olympus) and superfused at 25°C with a buffer containing 131 mM NaCl, 4 mM KCl, 1 mM CaCl₂, 1 mM MgCl₂, 10 mM glucose, and 10 mM HEPES at pH 7.4. The cells were field stimulated with suprathreshold voltage at a frequency of 0.5 Hz (unless otherwise stated), 3-ms duration, using a pair of platinum wires placed on opposite sides of the chamber and connected to an electrical stimulator (FHC Inc, Brunswick, Nebr). The myocyte being studied was displayed on a

computer monitor using an IonOptix MyoCam camera. An IonOptix SoftEdge software was used to capture changes in cell length during shortening and relengthening. Cell shortening and relengthening were assessed using the following indices: peak shortening (PS), indicative of the amplitude a cell can shorten during contraction; maximal velocities of cell shortening and relengthening ($\pm dL/dt$), indicative of peak ventricular contractility; time to PS (TPS), indicative of systolic duration; time-to-90% relengthening (TR90), indicative of diastolic duration (90% rather than 100% relengthening was used to avoid noisy signal at baseline concentration); and maximal velocities of shortening/relengthening, indicative of maximal velocities of ventricular pressure increase/decrease.

Intracellular Ca²⁺ fluorescence measurement

Myocytes were loaded with fura-2/AM (0.5 μ M) for 10 min, and fluorescence measurements were recorded with a dual-excitation fluorescence photomultiplier system (Ionoptix) 22. Myocytes were placed on an Olympus IX-70 inverted microscope and imaged through a Fluor 40 $\times$ oil objective. Cells were exposed to light emitted by a 75-W lamp and passed through either a 360- or a 380-nm filter, while being stimulated to contract at 0.5 Hz. Fluorescence emissions were detected between 480 and 520 nm by a photomultiplier tube after first illuminating the cells at 360 nm for 0.5 s then at 380 nm for the duration of the recording protocol (333-Hz sampling rate). The 360-nm excitation scan was repeated at the end of the protocol, and qualitative changes in intracellular Ca²⁺ concentration were inferred from the ratio of fura-2 fluorescence intensity (FFI) at two wavelengths (360/380). Fluorescence decay time was measured as an indication of the intracellular Ca²⁺ clearing rate. Both single and biexponential curve fit programs were applied to calculate the intracellular Ca²⁺ decay constant.

Generation of intracellular ROS

Production of cellular ROS was evaluated by analyzing changes in fluorescence intensity resulting from oxidation of the intracellular fluoroprobe 5- (and 6-) chloromethyl-2',7'-dichlorodihydrofluorescein diacetate (Molecular Probes, Eugene, Ore). In brief, isolated cardiomyocytes from each group were incubated with 25 μ M intracellular fluoroprobe 5- (and 6-) chloromethyl-2',7'-dichlorodihydrofluorescein diacetate in KRH buffer at 37°C for 30 min in the dark. Myocytes were then washed with warmed KRH buffer three times. The fluorescence intensity was measured using a fluorescent microplate reader at an excitation wavelength of 480 nm and an emission wavelength of 530 nm (Molecular Devices, Sunnyvale, Calif). Untreated cells showed no fluorescence and were used to determine background fluorescence. The final results were expressed as the ratio of the fluorescent intensity and protein content (25).

Protein carbonyl assay

The carbonyl content of protein was determined as described previously (26). Briefly, proteins were extracted and minced to prevent proteolytic degradation. Protein was precipitated by adding an equal volume of 20% trichloroacetic acid to protein (0.5 mg) and centrifuged at 11,000 $\times$ g for 5 min at 4°C. The CA solution was removed, and the sample was resuspended in 10 mM 4-dinitrophenylhydrazine solution. Samples were incubated at room temperature for 15 to 30 min. After addition of 500 μ L of 20% trichloroacetic acid, samples were centrifuged at 11,000 $\times$ g for 3 min at room temperature. The supernatant was discarded, the pellet was washed in ethanol-ethyl acetate and allowed to incubate at room temperature for 10 min. Samples were centrifuged again at 11,000 $\times$ g for 3 min at room temperature, and the ethanol-ethyl acetate steps were repeated twice more. The precipitate was resuspended in 6 M Guanadine HCl solution and incubated at 37°C for 60 min to dissolve pellets before being centrifuged again at 11,000 $\times$ g for 3 min at room temperature, and insoluble debris was

removed. The maximum absorbance (360 – 390 nm) of the supernatant was read against appropriate blanks, and the carbonyl content was calculated using the molar absorption coefficient of 22,000 L M⁻¹ cm⁻¹.

Caspase 3 assay

Caspase 3 is an enzyme activated during induction of apoptosis. The caspase 3 activity was determined according to the published method (27). Briefly, 1 mL of phosphate-buffered saline was added to a flask containing isolated ventricular myocytes before cells were scraped and collected in a microfuge tube. Cells were pelleted by centrifugation at 10,000 × g at 4°C for 10 min. The supernatant was discarded, and the cells were lysed in 100 µL of ice-cold cell lysis buffer (50 mM HEPES, pH 7.4, 0.1% CHAPS, 1 mM dithiothreitol, 0.1 mM EDTA, 0.1% NP40). The assay for caspase 3 activity was performed in a 96-well plate. Each well contained 30 µL of cell lysate, 70 µL of assay buffer (50 mM HEPES, pH 7.4, 0.1% CHAPS, 100 mM NaCl, 10 mM dithiothreitol, and 1 mM EDTA), and 20 µL of caspase 3 colorimetric substrate Ac-DEVD-pNA (Sigma Chemicals, St. Louis, Mo). The 96-well plate was incubated at 37°C for 2 h, during which time caspase in the sample was allowed to cleave the chromophore p-NA from the substrate molecule. Absorbance readings were obtained at 405 nm, with the caspase 3 activity being directly proportional to the colorimetric reaction. Protein content was determined using the Bradford method (28).

Western blot analysis

Expression of the stress signaling molecules JNK, p38, and ERK; the apoptotic proteins Bax and Bcl-2; and the ER stress markers GRP78 and Gadd153 was assessed using Western blotting. In brief, left ventricular tissue was sonicated in a lysis buffer containing 10 mM Tris, 150 mM NaCl, 5 mM EDTA, 1% Triton X-100, and protease inhibitor cocktail, followed by centrifugation at 10,000 × g for 10 min, 4°C. The supernatant was transferred to a clean microtube, and protein was quantified spectrophotometrically using the Bradford protein assay (28). Equal amount (30 µg protein/lane) protein and prestained molecular weight marker (GIBCO, Gaithersburg, Md) were loaded onto 10% to 12% sodium dodecyl sulfate–polyacrylamide gels in a minigel apparatus (Mini-PROTEAN II; Bio-Rad, Hercules, Calif), separated, and transferred to nitrocellulose membranes (0.2-µm pore size; Bio-Rad). Membranes were incubated for 1 h in a blocking solution containing 5% nonfat milk in Tris-buffered saline–Tween 20 before being washed in Tris-buffered saline–Tween 20 and incubated overnight at 4°C with anti-ERK (1:1,000), anti-phospho-ERK (1:1,000), anti-JNK (1:1,000), anti-phospho-JNK (1:1,000), anti-p38 (1:1,000), anti-phospho-p38 (1:1,000), anti-Bax (1:1,000), anti-Bcl-2 (1:1,000), anti-GRP78 (1:1,000), and anti-Gadd153 (1:1,000) antibodies. Anti-ERK and anti-pERK antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, Calif). All other antibodies were obtained from Cell Signaling Technology (Beverly, Mass). After incubation with the primary antibodies, blots were incubated with either antimouse or antirabbit immunoglobulin G horseradish peroxidase–linked antibodies at a dilution of 1:5,000 for 60 min at room temperature. Immunoreactive bands were detected using the Super Signal West Dura Extended Duration Substrate (Pierce, Milwaukee, Wis). The intensity of bands was measured with a scanning densitometer (model GS-800; Bio-Rad) coupled with Bio-Rad personal computer analysis software 27. Glyceraldehyde-3-phosphate dehydrogenase was used as the internal loading control.

Statistical analysis

Data were mean ± SEM. Differences between groups was assessed using ANOVA, followed by Tukey post hoc test or Student t test wherever appropriate. A P value less than 0.05 was considered statistically significant.

RESULTS

Plasma IGF-1 levels, mechanical, and intracellular Ca²⁺ properties of cardiomyocytes

Six hours of acute treatment of LPS (4 mg kg⁻¹, i.p.) did not significantly affect body and heart weights in either FVB or IGF-1 transgenic mice (data not shown). As expected, IGF-1 transgenic mice displayed significantly elevated plasma IGF-1 levels compared with FVB mice. Acute LPS treatment failed to affect plasma IGF-1 levels in either IGF-1 or FVB mice. The plasma IGF-1 levels were $1,623 \pm 196$, $1,729 \pm 233$, $3,624 \pm 323$, and $3,175 \pm 643$ pg mL⁻¹ in FVB, FVB-LPS, IGF, and IGF-LPS groups, respectively ($P < 0.05$ between FVB and IGF-1 groups, regardless of LPS treatment, mean $\pm$ SEM, $n = 4$). Mechanical recording revealed comparable resting cell length in cardiomyocytes from all four groups of mice. However, cardiomyocytes from the LPS-treated FVB mice displayed significantly reduced PS and maximal velocity of shortening/relengthening ($\pm$ dL/dt) associated with prolonged TR90 and similar TPS compared with myocytes from control FVB mice. Although IGF-1 transgene did not exert significant effect on cardiomyocyte contractile mechanics, similar to previous report (14), overexpression of the growth factor significantly attenuated LPS-induced cardiomyocyte mechanical dysfunction (Fig. 1). To explore the potential mechanism(s) involved in IGF-1 elicited protection against LPS-induced cardiomyocyte contractile defect, intracellular Ca²⁺ homeostasis was evaluated using the fluorescence dye fura-2. Our results indicated reduced intracellular Ca²⁺ clearing rate (both single and biexponential decays) associated with unchanged resting and electrically stimulated rise in intracellular Ca²⁺ levels in LPS-treated FVB mouse cardiomyocytes, the effect of which was ablated by transgenic overexpression of IGF-1. Insulin-like growth factor 1 itself did not elicit any overt effect on intracellular Ca²⁺ properties (Fig. 2).

Effect of IGF-1 on LPS-elicited effects on ROS generation, protein damage, and apoptosis

Figure 3 reveals significantly enhanced ROS production, protein carbonyl formation, and caspase 3 activity in LPS-treated FVB murine cardiomyocytes, suggesting the presence of oxidative stress, protein damage, and apoptosis in response to LPS insult. In line with its effect on cardiomyocyte mechanics, IGF-1 transgene nullified LPS-induced ROS accumulation, protein damage, and apoptosis without eliciting any significant effects itself.

Stress signaling activation, apoptotic proteins, and ER stress

LPS challenge is associated with activation of stress signaling. Results shown in Figure 4 indicate activation of JNK and p38 MAPK but not ERK in myocardium after LPS insult. Although IGF-1 transgene itself did not affect the activation/phosphorylation status in these stress signaling molecules, it ablated LPS-induced activation of JNK and p38 without altering the profile of ERK phosphorylation. Protein expression of nonphosphorylated JNK, p38, and ERK was not affected by either LPS or IGF-1. Our result shown in Figure 5 further depicted significantly up-regulated expression of the proapoptotic protein Bax and the ER stress markers GRP78 and Gadd153 in the LPS-treated FVB group, the effect of which was nullified by the IGF-1 transgene. Expression of Bcl-2 was unchanged by either LPS or IGF-1, although the combination of the two significantly elevated myocardial expression of Bcl-2. IGF-1 transgene itself did not affect expression Bax, GRP78, and Gadd153.

Effect of exogenous recombinant IGF-1 on LPS-induced cardiomyocyte dysfunction in vitro

To explore if LPS-induced cardiomyocyte contractile dysfunction is cardiac-specific or mediated through nonspecific proinflammatory response, cardiomyocytes from normal FVB mice were cultured with a sublethal dose of *E. coli* LPS (1 μ g mL⁻¹)²³ for 4 to 6 h in the presence or absence of exogenous recombinant IGF-1 (50 nM). The resting cell length was unaffected by either LPS or IGF-1. In vitro LPS exposure significantly decreased PS and $\pm$ dL/

dt while prolonging TR90 without affecting TPS in a manner reminiscent of in vivo LPS treatment. Interestingly, the LPS-induced reduction in PS, $\pm dL/dt$ but not prolongation in TR90, was ablated by 50 nM IGF-1 supplementation. Insulin-like growth factor 1 itself did not affect the mechanical properties (Fig. 6). These results suggest that LPS-elicited cardiomyocyte defect and IGF-1–offered cardioprotection may be attributed, at least in part, to changes in intrinsic cardiomyocyte properties.

DISCUSSION

Our study revealed that cardiac-specific overexpression of IGF-1 rescued LPS-induced cardiac contractile dysfunction and intracellular Ca^{2+} mishandling. The IGF-1–induced cardiac protection against endotoxemia may be underscored by alleviation of ROS accumulation, protein damage, and apoptosis. Furthermore, overexpression of IGF-1 alleviated LPS-induced activation of p38 and ERK stress signaling, as well as elevated ER stress. We further observed that exogenous IGF-1 administration alleviates, to some extent, in vitro LPS exposure–induced cardiomyocyte mechanical dysfunction reminiscent of the in vivo setting. These findings favor a direct cardiomyocyte cellular response as opposed to a consequence of the proinflammatory response induced by LPS, although the latter cannot be fully excluded at this time. Because IGF-1 itself did not significantly affect cardiac contractile function in the absence of endotoxemia, its protective role against LPS-induced cardiac contractile dysfunction implicates its potential in the clinical management of cardiovascular complication in sepsis.

An ample of evidence has depicted dysregulated cardiac function in sepsis (4,9). Our results revealed reduced PS and $\pm dL/dt$, prolongation of relaxation duration (TR90) in the endotoxemic FVB murine cardiomyocytes, or after in vitro LPS exposure, consistent with the previous findings (4,9,29). Our results showed that IGF-1 significantly attenuated LPS-induced cardiac contractile dysfunction, slowed intracellular Ca^{2+} clearance, ROS accumulation, protein carbonyl formation, and apoptosis (both caspase 3 and Bax expression), indicating a possible contribution of facilitated intracellular Ca^{2+} extrusion, lessened intracellular ROS, and protein damage by the IGF-1 transgene. The fact that IGF-1 itself did not affect cardiomyocyte contractile and intracellular Ca^{2+} properties and all biochemical makers in non–LPS-treated mouse hearts suggested that excessive amount of this growth factor is not innately harmful to cardiac function. Our current observation of the beneficial effect of IGF-1 against endotoxemia-induced cardiac dysfunction is somewhat consistent with its protective role against cardiac dysfunction in diabetes mellitus and advanced aging (13,14,30). One somewhat surprising finding from our study was that LPS treatment significantly attenuated cardiomyocyte contractile capacity (PS) without affecting electrically stimulated rise of intracellular Ca^{2+} (ΔFFI). Such discrepant findings seem to favor a reduced myofilament Ca^{2+} + sensitivity under endotoxemia, consistent with previous reports of reduced myofilament Ca^{2+} + sensitivity in cardiomyocytes in response to septic shock (11,31,32). Our results demonstrated that IGF-1 ablated sepsis-elicited activation of p38 and JNK, but not ERK, consistent with the findings of enhanced ROS accumulation, apoptosis, and protein damage. Activation of the MAPK stress signaling pathways has been widely documented in sepsis-induced cell death, tissue damage, and organ dysfunction, including myocardial contractile dysfunction (9,33–35).

Insulin-like growth factor 1 is a peptide growth factor synthesized by a variety of cell types, including cardiomyocytes, and may act as an autocrine/paracrine factor (12). Insulin-like growth factor 1 regulates myocardial growth and function under both physiological and pathophysiological conditions. It improves myocardial function in postinfarction rat heart, in patients with chronic heart failure, and in healthy humans and experimental animals (12). In the IGF-1 transgenic model used in this study, expression of the IGF-1 transgene is restricted to myocardium but not other organs, including skeletal muscle, brain, ovary, liver, lung, kidney,

and spleen (20). Insulin-like growth factor 1 is secreted from cardiomyocytes, resulting in increased circulating plasma levels of this growth factor as reported previously (14,20). Although our current study failed to reveal any change in plasma IGF-1 levels probably due to the short LPS treatment duration (6 h), endotoxemia has been reported to be associated with insufficient circulating IGF-1 levels. Evidence has indicated that supplementation of the growth factor may improve the overall survival rate in patients with sepsis. Insulin-like growth factor 1 is believed to mediate, to a large extent, the action of growth hormone in cardiac growth and contraction. It improves cardiac contractility, tissue remodeling, glucose metabolism, insulin sensitivity, and lipid profile (12). Data from our present study suggest that cardiac-specific overexpression of IGF-1 may compensate for the impaired intracellular Ca^{2+} homeostasis and myofilament Ca^{2+} sensitivity in sepsis. Interestingly, data from an in vitro study failed to reveal any beneficial effect of exogenous IGF-1 administration on LPS-induced prolongation in TR90, contrary to in vivo finding. Although the precise mechanism responsible for the discrepancy in diastolic duration (TR90) between in vitro and in vivo studies is not clear at this time, it is plausible to speculate likely involvement of certain proinflammatory machineries in LPS-triggered endotoxemic shock in the in vivo setting.

Our result revealed for the first time the presence of ER stress in myocardium after LPS-induced endotoxemia. Endoplasmic reticulum is an extensive intracellular membranous network involved in Ca^{2+} storage, Ca^{2+} signaling, glycosylation, and trafficking of membrane and secretory proteins. Sepsis has been shown to perturb these processes in the lung, thus creating a condition defined as ER stress (18). Recent evidence has indicated that ER stress contributes to neurodegenerative disorders, diabetes, and I/R-induced heart damage (36,37). Three different classes of ER stress transducers have been identified, namely, inositol-requiring protein 1, the protein kinase RNA-like ER kinase (PERK)-translation initiation factor eIF-2 α pathway, and activating transcription factor 6. Each of the three ER stress transducers governs a distinct arm of ER stress induced unfolded protein response (UPR) (37). Data from our current study revealed up-regulation of the UPR target proapoptotic protein Gadd153, a marker for PERK activity in the UPR (38). Our data also revealed LPS also up-regulated the ER chaperone GRP78 (also known as BiP), which directly interacts with all three ER stress sensors, PERK/eIF-2 α , activating transcription factor 6, and inositol-requiring protein 1, and maintains them in inactive forms (37). It is believed that up-regulation of GRP78 is pivotal for cell survival to facilitate folding and assembly of ER proteins and prevents them from aggregation during ER stress (37). Our observation that IGF-1 transgene nullified LPS-induced increase in GRP78 and Gadd153 suggests contribution of endotoxemia to myocardial ER stress. Although our study may not directly explain the interplay between cardiac dysfunction and ER stress after septic shock, ER stress has been shown to trigger apoptosis in I/R injury, which may be ablated by antioxidant treatment (39). This seems to be in line with the beneficial effect of antioxidants against sepsis (10). Our recent preliminary data indicated that ER stress directly leads to cardiomyocyte contractile dysfunction (19), supporting a role of ER stress in sepsis-induced cardiac dysfunction.

Experimental limitations

Although our current data have displayed some promises of IGF-1 transgene in the antagonism against sepsis-induced cardiomyocyte contractile dysfunction, it is not practical to overexpress IGF-1 for treatment of sepsis. Our data suggest that the artificial “jack-up” of the antioxidant defense using IGF-1, a potent antioxidant (12), may help compensate for reduced antioxidant defense in sepsis. In addition, it is worth mentioning that LPS-induced sepsis has significant limitations as a model for more clinically relevant sepsis. Other models such as the use of cecal puncture and ligation should be considered for a better understanding of the role of IGF-1 in sepsis-induced cardiac dysfunction and other organ complications.

In conclusion, our study revealed that cardiac specific overexpression of IGF-1 rescues LPS-induced cardiomyocyte mechanical dysfunction and intracellular Ca²⁺ mishandling, possibly through lessened ROS accumulation, protein damage, and apoptosis. It should be stated that other known beneficial effect of IGF-1, including antioxidant property, should not be ruled out at this time. These data have convincingly demonstrated the clinical potential of IGF-1 in the clinical management of endotoxemia-associated cardiac dysfunction.

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Fig. 1.

Contractile properties of cardiomyocytes from FVB and cardiac-specific IGF-1 overexpression transgenic mice treated with or without LPS (4 mg kg⁻¹, i.p.) for 6 h. A, Representative cardiomyocyte shortening traces from FVB mice treated with or without LPS. B, Representative cardiomyocyte shortening traces from IGF mice treated with or without LPS. C, Resting cell length. D, Peak shortening (normalized to resting cell length). E, Maximal velocity of shortening (+dL/dt). F, Maximal velocity of relengthening (-dL/dt). G, Time-to-PS. H, Time-to-90% relengthening (mean ± SEM, n = 63 – 66 cells from four mice in each group, **P* < 0.05 vs. FVB [without LPS treatment] group; †*P* < 0.05 vs. FVB-LPS group).

Fig. 2. Intracellular Ca^{2+} transient properties of cardiomyocytes from FVB and cardiac-specific IGF-1 overexpression transgenic mice treated with or without LPS (4 mg kg^{-1} , i.p.) for 6 h. A, Representative cardiomyocyte fura-2 transients from FVB mice treated with or without LPS. B, Representative cardiomyocyte fura-2 transients from IGF mice treated with or without LPS. C, Resting FFI. D, Electrically stimulated Δ FFI. E and F, Single and biexponential intracellular Ca^{2+} transient decay rate (mean $\pm$ SEM, $n = 33 - 40$ cells per group from four mice in each group, * $P < 0.05$ vs. FVB [without LPS treatment]; # $P < 0.05$ vs. FVB-LPS group).

Fig. 3.

Reactive oxygen species production, protein carbonyl formation, and caspase 3 activity in cardiomyocytes from FVB and cardiac-specific IGF-1 overexpression transgenic mice treated with or without LPS (4 mg kg⁻¹, i.p.) for 6 h. A, Intracellular ROS production using dichlorofluorescein fluorescence detection. B, Protein damage evaluated by protein carbonyl formation. C, Apoptosis assessed by caspase 3 activity (mean $\pm$ SEM; n = 5 – 7 mice per group, **P* < 0.05 vs. FVB [without LPS treatment]; #*P* < 0.05 vs. FVB-LPS group).

Fig. 4. Western blot analysis exhibiting expression of phosphorylated JNK, p38, and ERK in ventricles from FVB and cardiac-specific IGF-1 overexpression transgenic mice treated with or without LPS for 6 h. A, Representative gel blotting depicting total and phosphorylated JNK, p38, and ERK expression. B, Phospho-JNK-to-JNK ratio. C, Phospho-p38-to-p38 ratio. D, Phospho-ERK-to-ERK ratio (mean ± SEM; n = 5 – 6 samples per group, * $P < 0.05$ vs. FVB [without LPS treatment], # $P < 0.05$ vs. FVB-LPS group).

Fig. 5.

Western blot analysis exhibiting expression of the apoptotic protein Bax and Bcl-2, as well as the ER stress markers GRP78 and Gadd153, in ventricles from FVB and cardiac-specific IGF-1 overexpression transgenic mice treated with or without LPS for 6 h. A, Bax. B, Bcl-2. C, GRP78. D, Gadd153. Representative gel blotting depicting Bax, Bcl-2, GRP78, and Gadd153 using specific antibodies (inset; mean $\pm$ SEM; $n = 5 - 6$ samples per group, * $P < 0.05$ vs. FVB [without LPS treatment], # $P < 0.05$ vs. FVB-LPS group).

Fig. 6.

Contractile properties of cardiomyocytes from FVB mice treated with or without LPS (1 µg mL⁻¹) for 4 to 6 h *in vitro* in the presence or absence of IGF-1 (50 nM). A, Resting cell length. B, Peak shortening (normalized to cell length). C, Maximal velocity of shortening (+dL/dt). D, Maximal velocity of relengthening (-dL/dt). E, Time-to-PS. F, Time-to-90% relengthening (mean ± SEM, n = 39 – 40 cells per group, **P* < 0.05 vs. control; †*P* < 0.05 vs. LPS).