Endotoxin infusion in rats induces apoptotic and survival pathways in hearts

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McDonald, Treena E., Michelle N. Grinman, Chris M. Carthy, and Keith R. Walley. Endotoxin infusion in rats induces apoptotic and survival pathways in hearts. Am J Physiol Heart Circ Physiol 279: H2053–H2061, 2000.—Inflammatory mediators of sepsis induce apoptosis in many cell lines. We tested the hypothesis that lipopolysaccharide (LPS) injection in vivo results in induction of early apoptotic and survival pathways as well as evidence of late-stage apoptosis in the heart. Hearts were collected from control rats and at 6, 12, and 24 h after LPS injection (4 mg/kg). Activation of an apoptotic pathway was identified by a 1,000-fold increase in caspase-3 activity at 24 h (P < 0.05). Confirmation of these results occurred when terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) staining identified myocardial cells undergoing DNA fragmentation with significant levels at 24 h post-LPS injection. LPS also caused early proapoptotic mRNA (Bax) to increase (16% at 24 h, P < 0.05), whereas the Bax protein initially decreased (35% at 6 h, P < 0.05) and then returned to baseline values by 24 h. Six hours after LPS injection, Bcl-2 (early prosurvival) mRNA levels increased, whereas its protein levels decreased (70%, P < 0.05) and then returned to baseline levels by 24 h. Mitochondrial cytochrome c levels decreased, suggestive of mitochondrial involvement. Thus involvement of proapoptotic and prosurvival pathways in the heart occurs during a septic inflammatory response.

myocardium; sepsis; inflammation; apoptosis

APOTOSIS IN THE HEART has been documented during development as well as in disease states such as myocardial infarction, congestive heart failure, and ischemia reperfusion (3, 12, 23, 33). Thus apoptotic pathways can be activated in cardiomyocytes under specific conditions, i.e., by inflammatory mediators. For example, tumor necrosis factor-α (TNF-α) has the ability to activate apoptotic pathways in many cell lines via TNF receptor type 1 (TNFR1). TNFR1 is expressed on cardiac myocytes (17). Indeed, TNF-α induces apoptosis in cardiac myocytes in vitro (29). LPS-challenged cardiac myocytes have recently been shown to undergo apoptosis via LPS induction of myocyte TNF-α production and release (7). In addition, cytokine stimulation of inducible nitric oxide synthase (iNOS) resulting in nitric oxide production by the myocyte itself, by endothelial cells, and by leukocytes sequestered within the heart (11, 35) may also contribute to apoptosis (5, 9). Thus several sepsis mediators could activate apoptotic pathways in the heart. Whereas the in vitro experiments suggest the possibility of extensive apoptosis during sepsis and inflammatory states, this is not observed in humans and animals where myocardial dysfunction of sepsis is transient. The extent of myocardial apoptosis in whole animal models of sepsis is not known, and the mechanisms in vivo that may promote or prevent extensive myocardial apoptosis have not been elucidated.

Early proapoptotic proteins (i.e., Bax) are countered by early prosurvival proteins (i.e., Bcl-2) so that the balance between these competing activities determines cell fate (2). Stimuli that activate apoptotic pathways are associated with survival pathway counter regulation. For example, TNF-α and nitric oxide both induce apoptotic and survival pathways. Whether sepsis-induced activation of these pathways in the heart favors apoptosis or survival is unknown. Furthermore, the time course of activation of apoptotic and survival pathways in the heart after a septic stimulus is unknown.

We postulated that during sepsis, proapoptotic pathways are activated in the heart. Furthermore, we postulated that prosurvival pathways are also activated. As a result, end-stage apoptosis may be limited. To test these hypotheses, we used an acute lipopolysaccharide (LPS) model of sepsis in rats. Activation of apoptotic pathways in the heart was investigated by measuring the heart’s caspase-3 enzymatic activity and its apoptotic index as identified by terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL). We further tested for evidence of activation of early steps in both apoptotic and survival pathways by measuring relative levels of Bax and Bcl-2 mRNA and protein expression. We also investigated the temporal association of these pathways with myocardial function.
METHODS

These experiments conform to National Institutes of Health guidelines for the care and handling of animals and were approved by the University of British Columbia Animal Ethics Board.

Septic rat model. Male Sprague-Dawley rats (250 g) were given an intraperitoneal injection of 1 ml phosphate-buffered saline (PBS) or 4 mg/kg of LPS diluted in 1 ml PBS. The control rats were euthanized at 24 h post-PBS injection unless otherwise stated. LPS-injected rats were euthanized at multiple time points up to 24 h postinjection. Hearts were rapidly excised, cross sectioned into 3- to 4-mm-thick slices that included both the right and left ventricle, and subsequently frozen in liquid nitrogen or fixed with 10% Formalin. Frozen samples were stored at −80°C until needed, and the fixed samples were embedded in paraffin.

Caspase-3 activity assay. Caspase-3 activity was evaluated by measuring relative DEVDase, or caspase, cleavage activity. This assay also detects caspase-7 activity. Total heart cell lysates (20 μl) were incubated with caspase-specific fluorescent substrate as described by Granville et al. (13). The lysates were incubated with lysis buffer without the protease inhibitors, containing 100 μM of the caspase substrate acetyl-Asp-Glu-Val-Asp-7-amino-4-methyl coumarin, or Ac-DEVD-AMC. The reaction mixture was incubated at 37°C for 2 h, and fluorescence was measured using an excitation wavelength of 380 nm and an emission wavelength of 460 nm with a CytoFluor 2350 fluorescent measuring system (Perseptive Biosystems, Burlington, ON, Canada). Background fluorescence was determined by using an equal volume of the protein lysis buffer mixed with the above peptide (Ac-DEVD-AMC).

TUNEL staining. TUNEL was carried out using TdT-FragEL DNA Fragmentation Detection Kit (Oncogene, Cambridge, MA). Control cells consisted of the positive and negative controls provided with the kit. Heart tissue treated with 0.1 mg/ml DNase and heart tissue incubated without TdT, respectively, were additional positive and negative controls. TUNEL staining was performed according to the manufacturer’s instructions. A single-blinded observer determined the degree of apoptosis.

RNA isolation. RNA was extracted from one section of each rat heart using phenol-chloroform. The heart section was homogenized in RNase-free nucleic acid extraction buffer, rat heart using phenol-chloroform. The heart section was immediately placed in RNase-free water. Quantification of the RNA concentration in this solution was then adjusted to 2.5 μg/μl.

RT-PCR. Five micrograms of mRNA were reverse transcribed with the use of six units of SuperScript II reverse transcriptase (GIBCO BRL, Burlington, ON, Canada): 12.5 ng/μl oligo [dT]12-18, and 500 μM of each dNTP in an 1 U RNasin solution (Promega, Madison, WI), 5 μM 1,4-dithiothreitol, and 1× PCR buffer (20 mM Tris·HCl, pH 8.4; 50 mM KCl; 10, or 20 mM MgCl2 for amplification of Bax or Bel-2, respectively; 20 μM of each primer; and 1 U Taq DNA polymerase (GIBCO BRL) in a 20-μl reaction volume. The reaction for each gene of interest was performed with each gene of interest with its corresponding MgCl2 concentration and specific thermal cycle program. PCR mixtures used these conditions for Bax amplification: 38 cycles of 95°C for 30 s, 57°C for 40 s, and 72°C for 25 s followed by one cycle of 72°C for 6 min. The PCR thermal cycle conditions for Bel-2 were identical to Bax with two changes: the annealing temperature was 62.1°C for 45 s, and the extension was for 30 s. PCR conditions were confirmed to be within the linear range for the respective cDNA amplification. PCR products were identified by electrophoresing 10 μl of the PCR mixture on a 2% agarose gel in Tris-acetate buffer. The membranes were then washed, dried, and stored at room temperature.

Gel isolation of Bcl-2. Two milligrams of heart protein were incubated with 50 μl of agarose Protein A beads overnight at 4°C. The supernatant was removed, and the beads with bound protein were resuspended in 250 μl of loading buffer and heated as above.

Cell fractionation. Freshly isolated cardiac myocytes were resuspended in ice-cold lysis buffer at a concentration of 0.5 g tissue/ml of lysis buffer. The homogenized sample was gently rotated for 30 min and subsequently sonicated and centrifuged at 10,000 g for 10 min with all steps being carried out at 4°C or on ice. The supernatant was collected and stored at −80°C. Protein lystate (50 μg) was prepared in loading buffer consisting of 62.5 mM Tris·HCl, pH 6.8, 10% (vol/vol) glycerol, 2% (wt/vol) SDS, 10% (vol/vol) 2-mercaptoethanol, and 0.05% (wt/vol) bromphenol blue and was heated at 95°C for 4 min for SDS-PAGE analysis.

Isolation of Bcl-2. Two milligrams of heart protein were incubated with 50 μl of agarose Protein A beads overnight at 4°C. The supernatant was removed, and the beads with bound protein were resuspended in 250 μl of loading buffer and heated as above.

Protein isolation. One frozen section from each rat heart was homogenized in ice-cold lysis buffer at a concentration of 0.5 g tissue/ml of lysis buffer. The homogenized sample was gently rotated for 30 min and subsequently sonicated and centrifuged at 10,000 g for 10 min with all steps being carried out at 4°C or on ice. The supernatant was collected and stored at −80°C. Protein lystate (50 μg) was prepared in loading buffer consisting of 62.5 mM Tris·HCl, pH 6.8, 10% (vol/vol) glycerol, 2% (wt/vol) SDS, 10% (vol/vol) 2-mercaptoethanol, and 0.05% (wt/vol) bromphenol blue and was heated at 95°C for 4 min for SDS-PAGE analysis.
determined concentration, prepared in blocking solution, for 1 h. The Bax antibody, rabbit anti-rat (Oncogene), was used at 5 μg/ml. For Bcl-2 and cytochrome c, a monoclonal mouse anti-rat antibody was used at 1 μg/ml (Transduction Laboratories, Lexington, KY) and at 0.5 μg/ml (Pharmlingen, Mississauga, ON, Canada), respectively. Cytochrome oxidase (COX) subunit I involved an overnight incubation at 4°C with an antibody concentration of 0.5 μg/ml (Molecular Probes, Eugene, OR). The membrane was then washed with PBS + 0.1% Tween 20 (Sigma) for three 5-min washes and one 15-min wash. A secondary antibody was then added, composed of either horseradish peroxidase (HRP)-goat anti-rabbit IgG (0.67 μg/ml, UBI, Lake Placid, NY) or biotinylated horse anti-mouse IgG, rat absorbed (2 μg/ml, Vector Laboratories, Burlingame, CA). The incubation period of the secondary antibody was 30 min (rabbit) or 60 min (horse). The membrane was then washed as described earlier. Membranes probed with horse anti-mouse were incubated with 0.2 μg/ml strepavidin-HRP for 30 min at room temperature. Western blots were developed with the use of an enhanced chemiluminescence film (ECL, Amersham, Bucks, UK) following the manufacturer’s instructions, and the membranes were exposed to ECL Hyperfilm and developed.

**Isolation of cardiac myocytes.** In separate experiments, we measured fractional shortening of cardiac myocytes isolated from rats at multiple time points after LPS or PBS injection. Myocytes were isolated by collagenase digestion as previously described (37). After collagenase digestion of the heart, cardiac myocytes were washed once for the purpose of removing noncardiac myocytes. Thus the collected cell population consisted of >99% cardiac myocytes.

**Measurement of cardiac myocyte contractile function.** Myocyte contractile function was measured as previously described (37). Briefly, myocytes were considered viable if they demonstrated a characteristic rod shape without cytoplasmic blebbing. This morphometric assessment of viability was confirmed in a subset of experiments with trypan blue exclusion. Specifically designed platinum electrodes were lowered into each well in the 96-well plate, and the cardiac myocytes were electrically stimulated at 45 V (2.2-ms duration, 25 Ω resistance; S48 Stimulator, Grass Instruments, W. Warwick, RI) during videomicroscopy recording (Sony SLV-760HF). Still frames from the video recording of systolic and diastolic myocytes were captured for computer analysis. Fractional shortening was calculated as

\[
\% \text{ fractional shortening} = \frac{\text{diastolic length} - \text{systolic length}}{\text{diastolic length}}
\]

**Ex vivo heart functional studies.** The hearts were removed 6 h after the rats were injected with either LPS or PBS. The excised hearts were immediately placed in ice-cold Krebs-Henseleit solution, excess tissue was removed, the heart was hung on a Langendorff perfusion system, and the pulmonary artery was cut. Hearts were perfused using 37°C oxygenated Krebs-Henseleit solution at 65–75 mmHg of pressure and were maintained at a heart rate of 240–320 beats/min. A compliant latex balloon was inserted into the left ventricle of the heart through the mitral orifice. Hearts were allowed to stabilize for 15 min before the contractility measurement. Left ventricular pressure (LVP) was measured using a transducer (RayTech Instruments, Vancouver, BC, Canada). To measure contractility, we filled the balloon by using a constant-infusion pump (Havard Apparatus) at a rate of 200 μl/min from an initial volume of ~10 μl to a maximum volume of ~35 μl. Digitally captured LVP was then plotted against left ventricular volume, and peak systolic pressure-volume relationship was used as the best available measure of ventricular contractility independent of preload and afterload.

**Statistical analysis.** We tested for differences over time compared with control using an ANOVA. When a significant difference was found (P < 0.05), we identified specific differences using Student's t-tests corrected for multiple comparisons using a sequentially rejective Bonferroni test procedure.

**RESULTS**

To determine whether a key apoptotic pathway was activated in the LPS-exposed heart, caspase-3 enzymatic activity was measured in the whole heart. After LPS treatment, caspase-3 activity, as measured by cleavage of DEVD, continuously increased with maximum levels 1,000-fold greater (P < 0.05) than that produced by the control group at 24 h (Fig. 1).

To determine whether this degree of caspase-3 activation led to end-stage apoptosis or whether survival pathways limited this progression, we quantified TUNEL staining within the heart. With the use of TUNEL, no positive staining was seen in the viable control cells (provided with the kit) or in control heart tissue. Apoptotic cells within the LPS-treated hearts were seen at all time points (Table 1 and Fig. 2). These apoptotic cells were seen as single cells and were present in both ventricles. A significant increase in TUNEL staining was observed at 24 h post-LPS injection compared with all other groups (P < 0.05) (Table 1).

The mitochondria-associated Bcl-2 family was investigated as a possible upstream pathway that would regulate caspase-3 activation via the mitochondria. Specifically, we chose Bax as a representative proapoptotic protein and Bcl-2 as a representative prosurvival protein. Bax mRNA levels increased after LPS injection relative to control (Fig. 3A). Bax mRNA expression was maximum at 24 h post-LPS injection and was significantly greater than the control group (P < 0.05). Bax protein was expressed by the heart in all groups (Fig. 3B). Bax protein expression initially decreased significantly from control baseline to 6 h. At 12 h, Bax protein expression continually increased above that at 6 h, resulting in a return to control values by 24 h.

![Fig. 1. Effect of liposaccharides (LPS) on heart caspase-3 activity as measured by caspase (DEVase) activity. Data are expressed as means ± SE. *P < 0.05 compared with control; n, number of hearts.](http://ajpheart.physiology.org/)
Thus regulation and expression of this early proapoptotic protein are altered in the heart by LPS injection. LPS produced a different mRNA profile for Bcl-2 compared with Bax (Fig. 4A). Heart Bcl-2 mRNA levels increased at 6 h post-LPS injection compared with controls (P, 0.05) and then returned to control levels by 24 h. Bcl-2 protein showed a similar pattern to Bax (Fig. 4B). Bcl-2 protein levels initially decreased substantially at 6 h post-LPS injection and then returned to baseline from the 6-h time point to the 24-h time point. Thus regulation and expression of this early prosurvival pathway protein are altered in the heart by LPS injection.

Whereas both Bax and Bcl-2 mRNA and protein changed in the heart after LPS injection, the pattern of expression is neither clearly proapoptotic nor prosurvival. One exception may be at the 6-h time point after LPS injection, when the ratio of Bax to Bcl-2 may favor apoptosis (Fig. 5). To determine whether Bax, in relation to Bcl-2, was associated with release of cytochrome c from mitochondria, we measured cytochrome c in mitochondrial and cytosol fractions of control and LPS-treated rat hearts at 6 h postinjection. To ensure no contamination between the mitochondria and the cytosol fraction, the mitochondrial-specific protein COX was measured (Fig. 6A). Only the mitochondrial fraction contained the COX protein, indicating no contamination. Cytochrome c levels were significantly lower in the mitochondrial fraction of the 6-h LPS-treated group compared with control, P < 0.05 (Fig. 6, A and B), indicating a loss of mitochondrial cytochrome c in the LPS group. There was no significant change in the cytosolic cytochrome c. The ratio of cytochrome c in the mitochondria compared with the cytosol decreased, but this decrease was not statistically significant (Fig. 6C).

To determine whether these biochemical changes were temporally associated with cardiac functional changes, we measured both isolated cardiac myocyte fractional shortening and whole ventricle contractility using maximal elastance (E\textsubscript{max}). Fractional shortening for cardiac myocytes isolated from control rats was 19.6 ± 0.6%. Fractional shortening decreased by 29% 6 h after LPS injection (P < 0.01), indicative of myocardial dysfunction. Myocardial function then improved toward the control value, P < 0.05 compared with control (Fig. 7A). This decrease in myocardial contractility was confirmed by ex vivo functional studies, using the 6-h time point as a representative group (Fig. 7B). Ventricular contractility, as measured by $E_{\text{max}}$, decreased 54% after 6 h of LPS exposure compared with control, $P < 0.01$ (Fig. 7B).

**DISCUSSION**

In this rat LPS model of sepsis, we found evidence of involvement of both proapoptotic and prosurvival pathways as early as 6 h after LPS injection. By 24 h, we found evidence of activation of later apoptotic pathways and evidence of end-stage apoptosis of myocardial cells using TUNEL staining. Evidence of involvement of apoptotic pathways was associated with a partially reversible decrease in cardiac myocyte fractional shortening. The maximal decrease in fractional shortening was temporally associated more closely with mitochondria-related apoptotic pathway changes than with end-stage apoptosis. The absolute number of end-stage apoptotic cardiac myocytes is likely insufficient to ac-

### Table 1. TUNEL results of LPS-treated hearts

<table>
<thead>
<tr>
<th>Time Post-LPS Injection, h</th>
<th>No. of TUNEL-Positive Cells Per Cross Section of Heart</th>
</tr>
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<tbody>
<tr>
<td>≤4</td>
<td>2.3 ± 0.8</td>
</tr>
<tr>
<td>8</td>
<td>2.7 ± 1.2</td>
</tr>
<tr>
<td>12</td>
<td>4.3 ± 3.8</td>
</tr>
<tr>
<td>24</td>
<td>10.3 ± 4.7*</td>
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Values are means ± SE. *P < 0.05. TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling; LPS, lipopolysaccharide.
count for myocardial depression of sepsis, but it is conceivable that apoptotic and survival pathways, and particularly their relationship to mitochondrial function, may contribute to myocardial dysfunction of sepsis and acute inflammation.

In our model of sepsis, caspase-3 activity increased in the heart, as demonstrated by the DEVDase activity assay, to levels 1,000-fold greater than controls. Caspase-3 activation is a key finding and confirms that at least one apoptotic pathway has been activated.
Myocytes potentially contribute to the observed increase in caspase-3 activity. In healthy hearts, myocytes, fibroblasts, and smooth muscle cells have detectable levels of caspase-3 (26). Caspase signaling is important in cardiac myocyte apoptotic pathways (43, 44). Caspase-3 also colocalizes with apoptotic myocytes during myocardial infarction (4). Caspase-3 DEVDase activity has also been documented in the in vitro myocyte (42). In addition, the presence of active caspase-3 has been identified during ischemia and reperfusion and has been linked with the associated apoptotic death of cardiac myocytes (20). Thus the cardiac myocyte is a likely contributor to the caspase-3 activity present in this LPS-treated rat model.

Evidence of end-stage apoptosis within the heart comes from TUNEL staining. We observed increased apoptosis of myocardial cells with increasing time post-LPS injection. These apoptotic cells are found in both right and left ventricles of the heart. However, most cells within the heart did not demonstrate end-stage apoptosis. Therefore, a number of survival pathways may play an important role.

In our model of sepsis, early apoptotic and early survival proteins of the Bcl-2 family were investigated as possible regulators of subsequent caspase-3 activity. Six hours after the LPS injection, Bax and Bcl-2 protein expression declined and then gradually returned to control levels by 24 h. LPS directly or indirectly appears to be capable of regulating Bax and Bcl-2 protein levels independently of their mRNA. For instance, the half-life of Bax and Bcl-2 protein decreases in response to inflammatory stimuli (16). Specifically, caspases and other proteins involved in apoptosis can cleave and inactivate or downregulate the Bcl-2 family of proteins (15, 38). Other anti-apoptotic Bcl-2 family members within the heart like Mcl-1 and Bcl-xl may influence Bax levels (27, 28). Thus the early decrease in Bax and Bcl-2 that we observed may be explained by destruction or shortened half-life of these proteins. Thus Bax and Bcl-2 proteins are altered in the heart in this in vivo model of sepsis.

LPS also has an effect on regulating transcription of Bax and Bcl-2 genes or the stability of their mRNA as indicated by the changed Bax and Bcl-2 mRNA expression. The increase in Bax and Bcl-2 protein from the 6-h time point to 24-h post-LPS injection was likely contributed to by the increasing Bax mRNA from baseline to 24 h and by the increased Bcl-2 mRNA at the 6-h time point, respectively.

In this model of acute sepsis, both Bax and Bcl-2 protein levels declined and then rebounded so that the pattern of expression was neither clearly proapoptotic nor prosurvival. One exception occurred at 6 h after

Fig. 5. Effect of LPS on the heart’s Bax-to-Bcl-2 protein ratio. Data are from the densitometry results shown in Figs. 3 and 4. ∗P < 0.05 compared with control.

Fig. 6. Effect of LPS on cardiac myocyte cytochrome c levels. A: representation of a typical cytochrome c and cytochrome oxidase (COX) Western blot. M denotes mitochondrial fraction and C denotes cytosolic fraction. B: cytochrome c densitometry of the mitochondrial fraction in A. Densitometry results are adjusted for the amount of cell death caused by the myocyte isolation procedure and for the amount of protein loaded on the gel. C: ratio of cytochrome c in the mitochondria compared with that located in the cytosol based on the densitometory results shown in B. Data are expressed as means ± SE; n = 6 hearts. ∗P < 0.05 compared with control.
LPS injection, when Bax was predominantly favored over Bcl-2. To determine whether this change in the Bax-to-Bcl-2 ratio favored apoptosis, even though both protein levels had declined from baseline, we determined the net effect of Bax promoting and Bcl-2 inhibiting mitochondrial cytochrome c release (6, 14, 15, 39). Mitochondria have been shown to be frequently involved in apoptosis via the release of cytochrome c, which is regulated by the balance of Bax and Bcl-2 (6, 39). A pilot study identified that maximal changes in cytochrome c levels were occurring at 6 h after LPS injection (data not shown), which was in agreement with the Bax-to-Bcl-2 ratio. Thus cytochrome c levels were specifically investigated at 6 h after LPS injection. Cytochrome c levels were found to be lower within the mitochondrial fraction of the LPS-treated group compared with the control group by almost one-half. The ratio of mitochondrial to cytosolic cytochrome c decreased, but it was not statistically different between the LPS and the control group. Thus it is possible, but not certain, that the Bax-to-Bcl-2 ratio contributed to increasing caspase-3 activity via the release of cytochrome c.

It is not surprising that evidence of involvement of some apoptotic pathways is present in vivo, in view of previous in vitro results. LPS has been shown to induce end-stage apoptosis in cultured cardiac myocytes via induced myocyte production of TNF-α and activation of TNFR1 (7). Further support of TNFR1 involvement in myocyte apoptosis has been shown using TNF-α directly, antibodies to the TNFR1, or use of sphingosine, a known product of TNFR1 (29). In vitro studies have also shown the ability of TNF-α to induce apoptosis in endothelial cells and in smooth muscle cells (10, 41). Other inflammatory mediators of sepsis, including nitric oxide and reactive oxygen species, have been found to induce apoptosis in many cell types including myocytes (25, 36).

To investigate the correlation between activation of apoptotic and survival pathways with myocardial function, myocyte contractility was determined for each time point. LPS was found to maximally depress myocyte contraction at 6 h post-LPS treatment by 29%, which was confirmed by the 54% reduction in E_{max} for the intact heart. To show the relationship between fractional shortening and a volume-based measure such as E_{max}, we note that for a sphere a 25% fractional shortening of diameter translates into a 58% reduction in volume ejection at the same pressure. Half of the decrease in fractional shortening recovered by 12 h post-LPS treatment. These results agree with other investigators (1, 18, 19, 21, 32) who have used animal models that have demonstrated myocardial dysfunction after endotoxin infusion. The decrease in function observed here is temporally associated with the activation of apoptotic and survival pathways. However, these observations do not demonstrate a causal link.

Apoptosis is found in association with myocardial dysfunction in a number of clinical heart disease states such as ischemia-reperfusion, myocardial infarction, and chronic heart failure (12, 31, 33, 34, 40). However, the relationship between heart dysfunction and apoptosis has not been fully investigated. Increased myocyte apoptosis has been associated with age and ventricular dysfunction (22). Furthermore, failing ventricles in spontaneously hypertensive rats show greater than four times the number of apoptotic cardiomyocytes than those seen in nonfailing hypertensive rat ventricles (30). Ventricular dysfunction in these studies likely required apoptosis of many more cardiac myocytes than we observed in this relatively short sepsis study. Yet apoptotic pathways may be linked to cardiac myocyte dysfunction in other ways.

It is interesting to note that the maximal decrease in fractional shortening we observed after LPS injection was temporally more closely correlated with mitochondria-related apoptotic events, such as decrease in Bax and Bcl-2 protein, increase in Bax and Bcl-2 mRNA, and loss of mitochondrial cytochrome c, than with the evidence of minimal end-stage apoptosis at 24 h. Thus it is interesting to speculate that the effects of apoptotic pathways on mitochondrial function may be more important than end-stage apoptosis in contributing to myocardial dysfunction of sepsis.

In summary, we found involvement of apoptotic pathways in the heart in vivo after an LPS injection in...
rare. Apoptosis was evident 24 h after LPS injection as shown by the increased caspase-3 activity and the small degree of end-stage apoptosis. Both proapoptotic and prosurvival Bcl-2 family members were involved and may contribute by later cytochrome c release from the mitochondria. Apoptosis was found not to be selective to any particular region of the heart. We conclude that involvement of apoptotic and survival pathways occurs in the heart during a septic inflammatory response.

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