LPS pretreatment ameliorates peritonitis-induced myocardial inflammation and dysfunction: role of myocytes

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LPS pretreatment ameliorates peritonitis-induced myocardial inflammation and dysfunction: role of myocytes. Am. J. Physiol. 277 (Heart Circ. Physiol. 46): H885–H892, 1999.—Peritonitis induced by cecal ligation and puncture (CLP) produces a systemic inflammatory response that can be largely mitigated by pretreatment of the animals with lipopolysaccharide (LPS) tolerance. Although cells of myeloid origin and endothelial cells have been shown to contribute to the development of LPS tolerance, little is known regarding the potential role of parenchymal cells in this phenomenon. The major aim of the present study was to assess whether cardiac parenchymal cells (myocytes) contribute to the development of LPS tolerance. Six hours after induction of CLP rats were neutropenic and acidotic, the myocardium contained a leukocyte infiltrate [myeloperoxidase (MPO) activity was increased], and myocardial contractile function was impaired (left ventricular developed pressure was decreased). In animals that were pretreated with LPS these manifestations of sepsis were largely reversed. Further studies focused on the responses of cardiac myocytes to CLP and whether myocytes contributed to the development of LPS tolerance. Myocytes were isolated from rat hearts 6 h after induction of CLP. These myocytes 1) exhibited an impaired ability to shorten in response to pacing, 2) contained the nuclear transcription factor NF-κB in their nuclei, and 3) increased their surface levels of intercellular adhesion molecule-1 (ICAM-1), and 4) were hyperadhesive for neutrophils. All of these events did not occur in myocytes obtained from animals that were pretreated with LPS before induction of CLP. These findings indicate that LPS tolerance can be induced in myocytes with respect to polymorphonuclear leukocyte adhesion, presumably by an inability of CLP to mobilize NF-κB to the myocyte nuclei and, thereby, preventing an increase in surface levels of ICAM-1.

SEPSIS IS A GENERALIZED inflammatory response which involves organ systems remote from the locus of the initial infectious insult. The release of endotoxin [lipopolysaccharide (LPS)] from bacteria is generally believed to be the initial event in the development of sepsis (25). LPS activates vascular endothelium and induces the surface expression of adhesion molecules (e.g., proadhesive phenotype) (5, 9, 17). LPS also activates inflammatory cells of the myeloid lineage that subsequently amplify the inflammatory response by releasing various cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) (20, 33). This systemic inflammatory cascade results in polymorphonuclear leukocytes (PMN) sequestration in the lungs and adhesion to the endothelium of various systemic organs, e.g., the heart (3, 18). Subsequent PMN extravasation can lead to vascular dysfunction (protein leakage and edema formation) as well as parenchymal cell dysfunction. The importance of PMN-endothelial cell adhesive interactions in the development of organ dysfunction is exemplified by the protection against sepsis- or endotoxemia-induced tissue injury offered by monoclonal antibodies directed to adhesion molecules on PMN (CD18) (10, 28, 29).

The proinflammatory effects of LPS (and cytokines) requires activation of the nuclear transcription factor NF-κB in both endothelial cells and cells of myeloid origin (2, 23). In quiescent cells, NF-κB resides in the cytoplasm and is associated with an IκB inhibitory protein that prevents its translocation to the nucleus. LPS, TNF-α, IL-1β, as well as other cytokines induce a series of cellular events that result in the dissociation of NF-κB and IκB, thus allowing NF-κB to move into the nucleus. Activation and translocation of NF-κB to the nucleus results in the transactivation of a variety of genes that contribute to the systemic inflammatory response. In general, myeloid cells synthesize and secrete cytokines (e.g., TNF-α, IL-1β), whereas endothelial cells increase their surface expression of adhesion molecules [intercellular adhesion molecule-1 (ICAM-1), E-selectin]. These responses facilitate PMN-endothelial cell adhesion interactions, which subsequently lead to dysfunction of the affected organs.

Paradoxically, pretreatment of cells or animals with LPS renders them resistant to a subsequent LPS challenge (LPS tolerance) (33). For example, LPS-induced TNF-α secretion from a monocytic cell line can be substantially reduced by pretreating the cells with LPS (32). In rats, LPS-induced mortality and PMN accumulation within the heart can be largely ameliorated by pretreating the animals for several days with sublethal doses of LPS (3). LPS tolerance has also been demonstrated in humans (1). A pretreatment regimen with a neutral LPS derivative (monophosphoryl lipid A) decreased the systemic response (fever and tachycardia) to a subsequent LPS challenge. The growing body of evidence indicating that LPS tolerance can be induced in cells, animals, and humans has prompted the
suggestion that "LPS tolerance may be exploited for prophylaxis of severe sepsis in patients at risk" (33).

Numerous studies attempting to unravel the mechanisms involved in the development of tolerance to the proinflammatory effects of LPS have focused on cells of myeloid origin, i.e., macrophages and monocytes (33), and, to some extent, endothelial cells (15). There is no information available on the potential role of parenchymal cells in tolerance development with respect to inflammation. In the present study, we used an animal model of peritonitis, cecal ligation and perforation (CLP), to assess the role of the myocardial parenchyma (myocytes) in the development of LPS tolerance. To detect critical early events that occur after CLP and how they are altered during the development of LPS tolerance, we measured relevant variables 6 h after induction of CLP. With the use of this model, we show that myocardial myocytes contribute to the development of LPS tolerance and that the nuclear transcription factor NF-κB plays an important role.

METHODS

Animal Studies

Sprague Dawley rats (Charles River Canada, St. Constant, PQ) weighing 300–350 g were housed for 6 days in groups of six in standard cages and supplied ad libitum with laboratory chow and water. To study endotoxin tolerance, we gave a dosing regimen similar to one previously described (3). Briefly, the animals received daily intraperitoneal injections of increasing doses of endotoxin, Escherichia coli 026:B6 (Sigma, Mississauga, ON) over 3 consecutive days according to the following protocol: 0.15 mg/kg on day 1, 0.30 and 0.45 mg/kg on day 2, and 0.60 mg/kg on day 3. As a control, other animals received daily injections of an equal volume of saline.

CLP was then carried out on day 3 in both groups as previously described (13). Briefly, the animals were anesthetized 6 h after the LPS intervention on day 3 with halothane. Subsequently, the internal carotid artery and external jugular vein were cannulated under sterile conditions. These lines were tunneled subcutaneously to the back of the neck where they were attached to a swivel device. Animals then underwent CLP. After laparotomy, a ligature was placed around the cecum immediately distal to the ileocecal valve. This cecum was then incised along the antimesenteric border of the gut. After recovery from anesthesia, the following infusions were commenced: normal saline at 300–400 ml·kg⁻¹·day⁻¹ and fentanyl at 400 µg·kg⁻¹·day⁻¹. Heparin (400 U·kg⁻¹·day⁻¹) was administered to assure the patency of intravascular lines. Water and laboratory chow were available ad libitum. Sham-operated rats were used as controls for CLP. These animals were instrumented in a similar fashion to the endotoxin or saline pretreated group but did not undergo laparotomy. This animal study was reviewed and approved by the University of Western Ontario Committee on Animal Care.

Systemic variables. Mean arterial pressure was measured via the arterial catheter which was connected to a pressure transducer (Inflow, Baxter, Toronto, ON) and recorded with a multichannel, amplifier-recording system (Hewlett-Packard 78353A). Six hours after the CLP induction, arterial blood samples were withdrawn for complete cell count and analysis of blood gases and lactate.

Myocardial inflammation. As an index of PMN infiltration, myeloperoxidase (MPO) activity in the heart tissue was determined, as previously described (8). Briefly, after euthanasia, hearts were excised and placed in phosphate buffer and centrifuged at 6,000 g for 20 min at 4°C. The pellet was rehomogenized and sonicated for 10 s in 1 ml of 50 mM acetic acid (pH 6.0) containing 0.5% CETOH detergent. Twenty microliters of the prepared samples were used in reactions for MPO activity determined spectrophotometrically (650 nm) by measuring hydrogen peroxide-dependent oxidation of 3,3′,5,5′-tetramethylbenzidine. The results were expressed as absorbance per gram of tissue.

Myocardial function. Heart contractile function was assessed using the Langendorff isolated heart preparation (27). Briefly, after anesthesia and heparinization, the heart was rapidly excised, the ascending aorta was cannulated, and retrograde perfusion (10 ml/min) was initiated with Krebs-Henseleit solution containing (in mM) 120 NaCl, 4.8 KCl, 1.2 KH₂PO₄, 1.2 MgSO₄, 1.25 CaCl₂, 25 NaHCO₃ and 11 glucose; pH 7.4. Subsequently, the buffer was changed to one containing 2 mg/ml collagenase (Sigma, type I) and 0.1 mg/ml protease (Sigma, protease type XIV) and the heart perfused in a recirculating manner. The protease-containing buffer was washed out for 2 min with buffer A containing 0.2 mM CaCl₂. The heart was removed from the perfusion cannula and chopped with scissors in 15 ml of buffer containing 0.2 CaCl₂ and 25 mM KCl. The tissue was incubated at 37°C for 15 min, filtered through a 210-mesh nylon screen and the filtrate was centrifuged gently for 45 s. The buffer was aspirated off and the cells were resuspended in 50 ml of buffer containing 0.5 mM CaCl₂ for 10 min. The cells were centrifuged again and the buffer aspirated off. The final cell pellet was suspended in buffer A containing 1 mM CaCl₂. The cells were diluted to a concentration of ~100,000 cells/ml.

Myocyte function. The isolated myocytes were transferred to a Biophysics chamber (Biophysics Technologies, Baltimore MD) and seeded on the thermoregulated (36°C) stage of a Zeiss Axiosvert 35 inverted microscope. The cells were continuously superfused (bath volume 1 ml, flow rate 1 ml/min) with HEPES solution containing 1.8 mM CaCl₂ and field-stimulated with bipolar platinum electrodes with pulses of 5 ms duration at a frequency of 0.5 Hz using a Grass SD6 stimulator. Voltage was set at 4 V above threshold (12–25 V). Myocytes were allowed to stabilize for 30 min before pacing. Myocyte contractile activity was assessed by measuring their percent shortening. The cells were illuminated using a red filter (>600 nm) and the images were analyzed using a video.
edge-detector system (Colorado Video, Boulder, CO). The field was first calibrated at two distances with a graduated microscope slide and contractions of each myocyte were measured along the long axis. Each estimate of myocyte shortening represents the average of five consecutive contractions.

Myocyte-PMN adhesive interactions. Rat PMNs were isolated from rat blood using a two-component step gradient centrifugation approach using NIM-2 (Cardinal Association, Santa Fe). This procedure yields a PMN population that is 95% viable (trypan blue exclusion) and 90% pure (acetic acid-crystal staining).

Myocytes were plated in six-well plates and allowed to stabilize for 30 min (37°C) and then PMN added at a ratio of 1 myocyte to 10 PMNs. After 15 min of coincubation, PMN-myocyte adhesive interactions were assessed microscopically under static conditions with occasional shaking to differentiate adherent PMN from those making passive contact with myocytes. The number of PMN attached to myocytes were counted on at least 25 randomly chosen myocytes. Three experimental approaches were used in interacting myocytes with PMNN: 1) myocytes and PMN from CLP animals, 2) myocytes from sham animals and PMN from CLP animals, and 3) myocytes from CLP animals and PMN from sham animals.

Myocyte ICAM-1 expression. Isolated rat heart myocytes were fixed in 3% paraformaldehyde for 30 min at room temperature. After being washed with PBS, myocytes were treated with mouse anti-rat ICAM-1 antibody (1A2, 20 µg/ml) in the presence of 2% BSA for 1 h at room temperature. Subsequently, myocytes were washed and incubated with a biotinilated horse anti-mouse antibody (Pierce) (dilution 1:300) for 1 h. Myocytes were washed again and incubated with 3,3′,5,5′-tetramethylbenzidine (TMB) as a substrate. Cells (5 × 10^5) in total volume of 200 µl were used for the experiments. After incubation of myocytes for 5 min in substrate solution, they were centrifuged and 150 µl of supernatant was collected. The colorimetric reaction was stopped by adding 50 µl of 0.2 N H_2SO_4 and absorbance was read at 405 nm in microplate reader.

Myocyte NF-κB activation. Isolated myocytes were washed with cold PBS, centrifuged, and homogenized on ice in a Dounce homogenizer in a total volume of 5 ml of buffer E + 0.3% Nonidet P-40 (NP-40), 10 mM Tris (pH 8.0), 0.5 M MgCl_2, 5 mM dithiothreitol (DTT), 0.3 M sucrose, 1 µg/ml aprotonin, 1 µg/ml leupeptin, 1 mM PMSF). The homogenates were centrifuged at 500 g for 5 min, at 4°C. The supernatant was then removed, the pellets (nuclei) were resuspended in 5 ml of buffer E (buffer E− without Nonidet P-40) by several strokes in a Dounce homogenizer and sucrose concentration in homogenates was adjusted to 1.65 M by adding 2.32 g of a solid sucrose and allowing it to dissolve by gentle inversion of the tubes at 4°C. Subsequently, the homogenates were layered on a 3-ml sucrose cushion (2 M sucrose, 2 mM MgCl_2, 5 mM Tris-HCl, pH 7.5) and centrifuged at 25,000 × g for 1 h, at 4°C. The pellet fractions (nuclei) were resuspended in 30–50 µl of buffer C (22) (20 mM HEPES, 0.75 mM spermine, 0.15 mM spermine, 0.2 mM EDTA, 2 mM EGTA, 2 mM DTT, 20% glycerol, 1 mM PMSF, 4°C) and the ionic strength was adjusted to 0.4 M with NaCl. Samples were gently mixed on ice for 20 min. Finally, the samples were centrifuged for 10 min at 10,000 g (4°C) and the supernatants collected and saved as the nuclear protein fraction. The protein concentration was determined by the Bradford assay (4) and samples were stored at −80°C.

NF-κB activation was assessed by measuring NFκB in nuclear protein obtained from isolated myocytes using an electrophoretic mobility shift assay (EMSA) as previously described (19). The double-stranded oligonucleotide containing consensus (5′-AGGGACTTTCCGCTGGGACTTTC-3′) binding sites for NFκB (provided by Dr. T. Archer) were labeled with [γ-32P]ATP (Amersham Canada, Oakville, ON), by using T4 polynucleotide kinase (MBI Fermentas, Flamborough, ON) as previously described (19). One picomole of the labeled oligonucleotide was incubated with 5 µg of nuclear protein in the presence or absence of 50× excess of cold oligonucleotide for 30 min and the reaction mixture was then loaded onto native 5% PAGE and electrophoresed at 250 V in 0.5× Tris-borate-EDTA buffer. The dried gels then were exposed to X-ray films (Kodak) for 16 h in cassettes with intensifying screens.

Statistical Analysis

All results are presented as means ± SE. Statistical comparisons between the groups were carried out by ANOVA with an adequate post hoc test. P < 0.05 was accepted as statistically significant for all analyses.

RESULTS

Animal Studies

As shown in Table 1 induction of CLP (saline + CLP) had no effect on mean arterial blood pressure or arterial blood gases compared with the sham-operated animals. Induction of CLP did, however, produce neutropenia and systemic acidosis with an increase in lactate compared with the sham animals. These observations are consistent with the development of sepsis. Compared with saline pretreated animals exposed to CLP, LPS-pretreated animals exposed to CLP had a lower systemic lactate concentration and higher neutrophil counts. These latter observations indicate that the LPS-pretreated animals had a lesser degree of CLP-induced systemic manifestations of sepsis.

As shown in Table 1, LVDP and its first derivatives (i.e., +dP/dt and −dP/dt) were decreased 6 h after

Table 1. Systemic variables in sham, CLP, and LPS-pretreated CLP animals

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sham</th>
<th>Saline + CLP</th>
<th>LPS + CLP</th>
</tr>
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<tbody>
<tr>
<td>n</td>
<td>6</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>105±3</td>
<td>107±5</td>
<td>118±9</td>
</tr>
<tr>
<td>PaO_2, mmHg</td>
<td>86±6</td>
<td>81±9</td>
<td>70±10</td>
</tr>
<tr>
<td>Lactate, mmol/l</td>
<td>7.39±0.02</td>
<td>7.29±0.06*</td>
<td>7.38±0.01†</td>
</tr>
<tr>
<td>Neutrophils, 10^3/µl</td>
<td>2.25±0.15</td>
<td>0.42±0.08*</td>
<td>0.68±0.11†</td>
</tr>
</tbody>
</table>

Results are means ± SE. Saline + CLP, saline-pretreated animals subsequently exposed to CLP (cecal ligation and perforation); LPS + CLP, lipopolysaccharide (LPS)-pretreated animals subsequently exposed to CLP. MAP, mean arterial pressure; PaCO_2, oxygen arterial partial pressure. * P < 0.05 compared with sham animals; † P < 0.05 compared with saline + CLP.
induction of CLP (saline + CLP) compared with sham animals. LPS pretreatment largely prevented the effects of CLP on these variables (saline + CLP vs. LPS + CLP). Similarly, sham and LPS-pretreated CLP animals exhibited LVDP-preload and LV volume-preload relationships that were shifted upward and to the left of the saline pretreated CLP animals (Fig. 2). These observations indicate that contractile function of the heart was impaired in response to CLP and that LPS pretreatment protected the hearts from this CLP-induced dysfunction.

Myocyte Studies

Myocyte function. Ventricular myocytes from sham animals responded by shortening during electrical field stimulation (Fig. 3). Myocytes obtained from CLP animals had a significantly reduced degree of shortening (saline + CLP). The contractile function of myocytes obtained from LPS-pretreated animals exposed to CLP was at normal levels (sham vs. LPS + CLP).

Table 2. Left ventricular developed pressure and \( +\frac{dP}{dt} \) and \(-\frac{dP}{dt}\) in sham, CLP, and LPS-pretreated CLP animals

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Saline + CLP</th>
<th>LPS + CLP</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVDP (mmHg)</td>
<td>74 ± 7</td>
<td>54 ± 4*</td>
<td>78 ± 8†</td>
</tr>
<tr>
<td>( +\frac{dP}{dt} ) (mmHg)</td>
<td>4,606 ± 537</td>
<td>3,248 ± 365*</td>
<td>4,178 ± 378†</td>
</tr>
<tr>
<td>(-\frac{dP}{dt} ) (mmHg)</td>
<td>2,846 ± 359</td>
<td>2,126 ± 141*</td>
<td>3,426 ± 376†</td>
</tr>
</tbody>
</table>

Results are means ± SE (in mmHg); n = 7 in each group. Saline + CLP, saline-pretreated animals subsequently exposed to ceal ligation and perforation (CLP); LPS + CLP, LPS-pretreated animals subsequently exposed to CLP. LVDP, left ventricular developed pressure; \( +\frac{dP}{dt} \), first derivative of pressure development over time; \(-\frac{dP}{dt} \), first derivative of pressure relaxation over time. *P < 0.05 compared with sham animals; †P < 0.05 compared with saline + CLP.

Myocyte-PMN adhesion. When myocytes and PMN obtained from CLP-treated animals were interacted in an adhesion assay, there was an increase in PMN adhesion to myocytes (Fig. 4A). This hyperadhesion response was substantially diminished when PMNs and myocytes from CLP animals pretreated with LPS were used (saline + CLP vs. LPS + CLP). PMN obtained from CLP animals (saline + CLP) adhered more avidly to naive myocytes than PMNs obtained from sham animals (Fig. 4B). This hyperadhesive response was reversed when PMN from CLP animals pretreated with LPS were used (saline + CLP vs. LPS + CLP).
When myocytes from CLP animals were reacted with naive PMNs (from sham animals) there was an increase in PMN adhesion to myocytes (Fig. 4C). The adhesiveness of myocytes for PMN was dramatically reduced when myocytes were obtained from CLP animals that had been pretreated with LPS (LPS vs. saline vs. CLP).

Myocyte ICAM-1. Because previous studies indicated that LPS, TNF-α, or IL-1β can induce an increase in ICAM-1 expression on isolated cardiac myocytes and make them more adhesive for PMNs (24), we assessed whether myocyte ICAM-1 plays a role in the development of LPS tolerance in our model. As shown in Fig. 5, surface levels of ICAM-1 were increased on myocytes obtained from CLP animals (saline vs. CLP) compared with those obtained from sham animals. This increased level of ICAM-1 expression in response to CLP was significantly reduced when myocytes obtained from CLP animals pretreated with LPS were used.

Myocyte NF-κB. The nuclear transcription factor, NF-κB, appears to be intimately involved in the inflammatory process by transactivating the gene encoding ICAM-1 (23). Thus we assessed whether NF-κB activation and translocation to the nucleus was involved in the development of LPS tolerance in myocytes. As shown in Fig. 6, there was more NF-κB in the nuclei of myocytes obtained from CLP animals (saline vs. CLP) compared with those from sham animals. NF-κB was virtually undetectable in nuclei of myocytes obtained from CLP animals pretreated with LPS.

DISCUSSION

A recent study in mice demonstrated that polymicrobial sepsis induced by CLP increases mRNA levels for a variety of cytokines and chemokines in the lung and liver 6 h after CLP and that prophylactic administration of monophosphoryl lipid A (a nontoxic derivative of...
LPS) attenuated these responses (21). In the present study we focused on the heart and functional manifestations of CLP-induced sepsis. We show that 6 h after induction of CLP in rats, there was evidence of myocardial inflammation (Fig. 1) and contractile dysfunction (Table 2 and Fig. 2) and that LPS pretreatment ameliorates these cardiac manifestations of sepsis. The mechanisms involved in the development of this LPS-induced tolerance are not completely clear. Most studies have focused on the role of inflammatory cells of myeloid origin or endothelial cells in the development of LPS tolerance (15, 33). Although previous studies have shown that LPS tolerance to a subsequent LPS challenge can be induced in a parenchymal cell, i.e., myocytes (with respect to cell shortening) (14), the present study represents the first systematic evaluation of the role of myocytes in the development of LPS tolerance with respect to inflammation.

In the present study myocardial contractile activity was depressed by CLP-induced sepsis as evidenced by 1) a decrease in the LVDP and its first derivatives +dP/dt and −dP/dt (Table 2), as well as 2) a shift in the LVDP and LV volume-preload relationships downward and to the right (Fig. 2). These indexes were near normal in rats pretreated with LPS before induction of CLP (Table 2 and Fig. 2). In analogous in vitro experiments, isolated myocytes from rats subjected to CLP had a reduced ability to shorten in response to pacing (Fig. 3). The mechanisms responsible for this shortening defect are unclear. Previous studies indicate that the LPS-induced decrease in myocyte shortening is not associated with alterations in intracellular Ca²⁺ transients, indicating that a decrease in myocyte myofilament response to Ca²⁺ may be involved (31). A similar mechanism may be operative in the present study. Irrespective of the mechanisms involved in the depressed contractile activity of myocytes from CLP animals, the contractile activity of myocytes from CLP animals that had been pretreated with LPS were near normal, indicating that myocytes are an active participant in the development of LPS tolerance in vivo. These observations are consistent with a previous in vitro study showing that LPS-induced myocyte shortening can be prevented by a previous LPS challenge (14). Thus, the major significance of our observations is that LPS tolerance (with respect to myocyte shortening) can be induced to polymicrobial sepsis (CLP).

In the present study, when myocytes and PMNs isolated from CLP animals were reacted in an adhesion assay, there was a fivefold increase in PMN adhesion to myocytes (Fig. 4A). This hyperadhesive response was ameliorated when myocytes and PMNs from CLP animals that had been pretreated with LPS were reacted in the adhesion assay. Although these experiments demonstrate that LPS tolerance with respect to PMN-myocyte adhesion was induced, they do not allow for a delineation of the relative roles of myocytes and PMNs in this response. Our subsequent experiments using myocytes from CLP animals and PMNs from sham animals and vice versa, indicate that both cell types contribute to the development of LPS tolerance in this system.

PMN obtained from CLP-treated animals adhered to naive myocytes (obtained from sham animals). This observation is consistent with the observation that LPS-stimulated PMN are more adhesive to endothelium (30). In the present study, this hyperadherence response was no longer present if the PMN were obtained from CLP animals previously pretreated with LPS (Fig. 4B). This latter observation indicates that PMN contribute to the development of LPS tolerance in vivo. This is not entirely surprising considering the numerous observations that cells of myeloid origin can be rendered tolerant to LPS with respect to cytokine production (33) and that PMN obtained from LPS-tolerant rats are less adherent to nylon fibers (3). Nonetheless, our findings are the first to demonstrate that LPS tolerance can be induced in PMN with respect to adhesivity for myocytes.

Of further relevance to the present study is the role of myocytes in the development of LPS tolerance. Myocytes from CLP animals were more adhesive to naive neutrophils, whereas myocytes from CLP animals pretreated with LPS developed LPS tolerance with respect to PMN adhesion (Fig. 4C). These findings are the first to show that a parenchymal cell, i.e., cardiac myocyte, can develop LPS tolerance with respect to PMN adhesion.

Previous studies indicate that LPS, TNF-α, and IL-1β can induce canine myocytes to be more adhesive for PMNs (6, 24). The adhesion of PMN to cardiac myocytes results in an intracellular oxidative stress within the myocytes and myocyte dysfunction, i.e., contracture (6). This cytokine-induced 1) PMN adhesion and 2) PMN-induced injury to the myocytes is a result of an increased surface level of ICAM-1 on the myocytes (24), because both of these events can be prevented by monoclonal antibodies directed to ICAM-1 (6). In the present study, myocytes from CLP animals had an increased surface level of ICAM-1, a finding consistent with the aforementioned studies. More importantly, the surface levels of ICAM-1 were not altered in CLP animals pretreated with LPS. This latter observation indicates that the development of LPS tolerance in myocytes (with respect to PMN adhesion) is associated with a diminished ability of myocytes to increase their surface levels of ICAM-1 in response to LPS adhesion.

Increases in surface levels of ICAM-1 are dependent on protein synthesis apparently resulting from the transactivation of the ICAM-1 gene. Transactivation of the ICAM-1 gene is initiated by nuclear transcription factors, such as NFκB (19). In the present study, there were increased levels of NFκB in the nucleus of myocytes from CLP animals, indicating that NFκB had been activated and translocated to the nucleus. This is consistent with previous reports showing that exposing isolated neonatal cardiac myocytes to the cytokine, IL-1β (16), results in translocation of NFκB to the nucleus. The novel observation of the present study is that there was no detectible NFκB in the nucleus of myocytes obtained from CLP animals pretreated with...
LPS (Fig. 6). These latter observations indicate that the development of LPS tolerance in myocytes is associated with a lack of NFκB translocation to the nucleus in response to CLP. These observations are also consistent with a role for NFκB in the development of LPS tolerance in monocytes and macrophages where LPS-tolerant cells are unable to mobilize this nuclear transcription factor in response to further LPS stimulation (11, 12, 26).

In summary, the results of the present study indicate that parenchymal cells may contribute to the LPS-induced tolerance to polymicrobial sepsis in vivo. Specifically, our results support a role for cardiac myocytes in the development of LPS-induced tolerance to CLP-induced peritonitis. In a rat model 6 h after induction of CLP: 1) myocyte shortening is impaired, 2) myocyte NFκB is activated and translocates to the nucleus, 3) myocyte surface levels of ICAM-1 are increased and 4) myocytes are adhesive for naïve PMN. All of these events are prevented if the rats are pretreated with LPS before induction of CLP. A similar scenario may also occur in the parenchyma of other organs and contribute to the reduced PMN sequestration and infiltration in LPS-tolerant animals or humans subsequently exposed to sepsis. Thus development of therapeutic modalities for the amelioration of sepsis-induced multiple organ failure should also take into account the contribution of parenchymal cells to the development of LPS tolerance.

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