LPS induces late cardiac functional protection against ischemia independent of cardiac and circulating TNF-α

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Meng, Xianzhong, Lihua Ao, James M. Brown, Daniel R. Meldrum, Brett C. Sheridan, Brian S. Cain, Anirban Banerjee, and Alden H. Harken. LPS induces late cardiac functional protection against ischemia independent of cardiac and circulating TNF-α. Am. J. Physiol. 273 (Heart Circ. Physiol. 42): H1894–H1902, 1997.—Lipopolysaccharide (LPS) and tumor necrosis factor (TNF)-α independently induce cardioprotection against ischemia in the rat at 24 h after administration, suggesting that endogenously synthesized TNF-α may play a role in LPS-induced protection. The purposes of this study were 1) to delineate the time course of LPS-induced cardiac functional protection against ischemia and its relation with myocardial and circulating TNF-α profile, 2) to examine whether prior protein synthesis inhibition abrogates the protection, and 3) to assess the effects of TNF-α inhibition and neutralization on the protection. Rats were treated with LPS (0.5 mg/kg ip). Cardiac functional resistance to normothermic global ischemia-reperfusion was examined at sequential time points after LPS treatment in isolated hearts by the Langendorff technique. Myocardial and circulating TNF-α was determined by enzyme-linked immunosorbent assay at 1–24 h after LPS treatment. Protection was apparent at 24 h, 3 days, and 7 days but not at 2 or 12 h. Maximal protection at 3 days was abolished by cycloheximide pretreatment (0.5 mg/kg ip 3 h before LPS treatment). Increases in myocardial and circulating TNF-α preceded the acquisition of protection. Dexamethasone pretreatment (4.0 or 8.0 mg/kg ip 30 min before LPS treatment) abolished peak increase in myocardial TNF-α and substantially suppressed circulating TNF-α (54.3 and 85.9% inhibition, respectively) without an influence on the maximal protection. Similarly, maximal protection was not affected by TNF binding protein (40 or 80 µg/kg iv immediately after LPS treatment). The results suggest that LPS-induced cardiac functional protection against ischemia is a delayed and long-lasting protective response that may involve de novo protein synthesis. Although LPS-induced increase in myocardial and circulating TNF-α precedes the delayed protection, it may not be required for the delayed protection.

Enhanced cardiac resistance to ischemia observed at 24 h after exposure to LPS may require de novo synthesis of proteins involved either in the induction of the cardiac protective response or directly in the protective mechanisms. LPS induces the expression of tumor necrosis factor (TNF)-α in a variety of tissues including myocardium (10, 11), which involves both transcription and translation. Previous work from our (3) and other (7, 25) laboratories showed that exogenous TNF-α can induce cardiac protection against ischemia-reperfusion injury at 24 h after administration, indicating that endogenously synthesized TNF-α may play a role as a mediator in LPS-induced cardiac protection against ischemia. Although this hypothesis is intriguing, it needs supporting data that protein synthesis inhibition attenuates or abrogates this protection. Furthermore, the temporal relation of the induction of myocardial and circulating TNF-α by LPS with the acquisition of cardiac protection is critical for the determination of a potential causal relationship between these two responses to LPS. However, the temporal relation between these two responses has not been determined.

Glucocorticoids suppress LPS-induced tissue TNF-α production (8, 33) primarily through inhibition of nuclear factor-κB (1, 2). Thus glucocorticoid pretreatment prevents LPS-induced, TNF-α-mediated expression of inducible nitric oxide synthase (iNOS) (26), cardiac contractile depression (22), and shock (14). Furthermore, TNF binding proteins (TNFBP), functioning as TNF-α antagonists, can abolish the in vitro negative inotropic properties of TNF-α in cardiac myocytes (12) and protect against excessive TNF-α in vivo (32). It is unknown whether suppression of tissue TNF-α production with glucocorticoids or neutralization of circulating TNF-α with TNFBP has any influence on LPS-induced cardiac protection against ischemia. The present study was therefore initiated 1) to delineate the time course of LPS-induced cardiac functional protection against ischemia, 2) to examine whether pretreatment with a protein synthesis inhibitor, cycloheximide, attenuates or abrogates this protection, 3) to examine the temporal relation of the induction of myocardial and circulating TNF-α by LPS with the acquisition of this protection, and 4) to assess the effects of suppression of myocardial and circulating TNF-α with glucocorticoids and neutralization of circulating TNF-α with TNFBP on this protection.
MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats, body weight 300–350 g (Sasco, Omaha, NE), were acclimated in a quarantine room and maintained on a standard pellet diet for 2 wk before initiation of the experiments. All animal experiments were approved by the Animal Care and Research Committee, University of Colorado Health Sciences Center. All animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals [DHHS Publication No. (NIH) 85–23, Revised 1985, Office of Science and Health Reports, DRR/NIH, Bethesda, MD 20892].

Chemicals and reagents. Dexamethasone was purchased from Elkins-Sinn (Cherry Hill, NJ). Human TNFBP2 was a generous gift from Immunex. This TNFBP is a chimeric fusion protein (soluble TNF receptor:Fc, 102 kDa), consisting of a dimer of two molecules of human TNFBP2 linked by the Fc portion of the human immunoglobulin G1 (12). TNF-α assay kit was obtained from Genzyme (Cambridge, MA). LPS (from Salmonella typhimurium), cycloheximide, and other chemicals were obtained from Sigma Chemical (St. Louis, MO).

Experimental protocols. To examine the time course of LPS-induced cardiac functional protection against ischemia, rats were treated with a single dose of LPS (dissolved in bacteriostatic normal saline, 0.5 mg/kg ip) or bacteriostatic normal saline (0.4 ml ip). Their hearts were isolated at 2, 12, or 24 h, 3 days, or 7 days and subjected to normothermic global ischemia-reperfusion. Our previous study showed that LPS depresses cardiac contractility at 4–12 h after administration (24). Therefore, the time points chosen for this study represent those before, during, and after cardiac depression. Effects of protein synthesis inhibition on LPS-induced maximal cardiac protection were examined by the injection of cycloheximide (0.5 mg/kg ip) 3 h before LPS treatment (0.5 mg/kg ip). At 3 days after LPS treatment, hearts were isolated and subjected to normothermic global ischemia-reperfusion. Control rats were treated with cycloheximide alone (0.5 mg/kg ip). Hearts were isolated at 3 days after treatment and subjected to normothermic global ischemia-reperfusion. The cycloheximide dose has been shown to inhibit de novo protein synthesis in rat tissues by 75% over a period of 16 h (27). Our preliminary experiments suggest that this is the maximal cycloheximide dose tolerable by rats subsequently treated with LPS, i.e., without provoking mortality.

To examine the effect of LPS on myocardial and circulating TNF-α levels, rats were treated with a single dose of LPS (0.5 mg/kg ip) or bacteriostatic normal saline (0.4 ml ip) and killed at sequential time points from 1 to 24 h after the treatment. Blood was collected through the right atrium, and serum was prepared by centrifugation and stored at −70°C. Hearts were excised, and coronary blood vessels were flushed with 10 ml of phosphate-buffered saline (pH 7.4, 4°C) by retrograde perfusion through the aortic root. After the atria were removed, ventricular (both left and right) tissue was frozen in liquid nitrogen and stored at −70°C. Immediately before the TNF-α assay was performed, myocardium was homogenized with a tissue homogenizer (Tekmar, Cincinnati, OH) in 4 vols of phosphate-buffered saline (pH 7.4, 4°C). After centrifugation at 1,200 g at 4°C for 20 min, the supernatant was collected for TNF-α assay.

Effects of glucocorticoids on LPS-induced TNF-α production were examined by the injection of dexamethasone (4.0 or 8.0 mg/kg iv) 30 min before LPS treatment (0.5 mg/kg ip). At 1 h after LPS treatment, blood and ventricular tissue were collected. Serum and myocardial homogenate were prepared as described above for the TNF-α assay. The dexamethasone dose has been reported to be effective in inhibition of the in vivo induction of TNF-α (8, 33) and iNOS (16) by LPS. Moreover, our previous work indicated that dexamethasone at a dose of 4.0 mg/kg attenuates the in vivo myocardial depressive effect of LPS (22).

Effects of glucocorticoids on LPS-induced maximal cardiac protection were examined by the injection of dexamethasone (4.0 or 8.0 mg/kg iv) 30 min before LPS treatment (0.5 mg/kg ip). At 3 days after LPS treatment, hearts were isolated and subjected to normothermic global ischemia-reperfusion. Control rats were treated with dexamethasone alone (8.0 mg/kg iv). Hearts were isolated 3 days after treatment and subjected to normothermic global ischemia-reperfusion.

Effects of TNFBP on LPS-induced maximal cardiac protection were examined by the administration of a chimeric dimer of TNFBP2 (40 or 80 µg/kg iv, calculated circulating concn ~7.5 or 15 nmol/l blood) immediately after LPS treatment (0.5 mg/kg ip). At 3 days after LPS treatment, hearts were isolated and subjected to normothermic global ischemia-reperfusion. Control rats treated with TNFBP2 alone (80 µg/kg iv). Hearts were isolated 3 days after treatment and subjected to normothermic global ischemia-reperfusion. This chimeric dimer of TNFBP2 has been shown to be more effective in neutralizing TNF-α than monomeric TNFBP2 or TNFBP1. At a concentration of 11 nM, it completely neutralized TNF-α (25 PM) cytotoxicity to 159L-RE 3.5 cells. It also abolished TNF-α-induced contractile depression in isolated feline cardiac myocytes over a wide concentration range from 1.1 to 11 nM (12). In the preliminary experiments, we observed that intravenous administration of TNFBP at either dose (40 or 80 µg/kg) completely abolished cardiac contractile depression induced by LPS at the same dose applied in this study.

Measurement of hemodynamics. Two additional groups of rats were treated with LPS (0.5 mg/kg ip) or bacteriostatic normal saline (0.4 ml ip). They were anesthetized (50 mg/kg pentobarbital sodium ip) and heparinized (200 U heparin sodium ip) at 2, 4, 24, or 72 h after LPS treatment. The right femoral artery was cannulated with PE-50 polyethylene tubing (Becton-Dickinson, Parsippany, NJ). Heart rate and arterial pressure were recorded for 20 min with a computerized pressure amplifier-digitizer (MacLab/8, AD Instrument, Cupertino, CA and Macintosh Quadra 800, Apple Computer, Cupertino, CA).

Isolated heart perfusion and assessment of cardiac function. Cardiac function was determined by a modified isovolumetric Langendorff technique as described elsewhere (22–24) and expressed as coronary flow, left ventricular end-diastolic pressure (LVEDP), and left ventricular end-diastolic pressure (LVEDP). At the termination of the experiments, beating hearts were rapidly excised into oxygenated Krebs-Henseleit solution containing (in mmol/l) 5.5 glucose, 1.2 CaCl2, 4.7 KCl, 25 NaHCO3, 119 NaCl, 1.17 MgSO4, and 1.18 KH2PO4. Normothermic retrograde perfusion was performed with the same solution in an isovolumetric and nonrecirculating mode. The perfusion buffer was saturated with a gas mixture of 92.5% O2–7.5% CO2 to achieve Po2 of 450 mmHg, PCO2 of 40 mmHg, and pH of 7.4. Perfusion pressure was maintained at 70 mmHg. A latex balloon was inserted through the left atrium into the left ventricle, and the balloon was filled with water (0.18–0.28 ml) to achieve an LVEDP of 5–10 mmHg (at peak and flat portions of LVEDP-LVEDP curve). Wring wires were fixed to the right atrium, and the heart was paced at 6.0 Hz. Coronary flow was measured by collection of effluent from the pulmonary arteries. LVEDP and LVEDP were continuously recorded with the computerized pressure amplifier-digitizer. A three-way stopcock was mounted above the aortic cannula.
to create global ischemia. After 15 min of perfusion (equilibration), hearts were subjected to 25 min of normothermic global ischemia followed by 40 min of reperfusion. During ischemia, hearts were placed in a perfusate-filled organ bath chamber without pacing. Myocardial temperature was maintained at 37°C by circulation of warm water.

TNF-α assay. TNF-α levels in myocardium and serum were measured using an enzyme-linked immunosorbent assay (ELISA) system containing a hamster anti-mouse TNF-α antibody (cross-reaction with rat TNF-α). Recombinant murine TNF-α was used to construct a standard curve. Absorbance of standards and samples was determined spectrophotometrically at 450 nm using a microplate reader. Results were plotted against the linear portion of the standard curve.

Statistical analysis. Data are expressed as means ± SE. An analysis of variance was performed, and a difference was accepted as significant when P < 0.05 was verified by Bonferroni-Dunn test.

RESULTS

General effects of LPS treatment. The body weight of LPS-treated rats decreased from the baseline of 387 ± 6.8 to 366 ± 6.1 g by 24 h (P < 0.05). LPS-treated rats recovered their body weight at 7 days after treatment. LPS treatment also resulted in a slight increase in body temperature. The baseline rectal temperature of anesthetized rats was 37.2 ± 0.17°C. It increased to 38.0 ± 0.10°C at 4 h after injection of LPS (P < 0.05 vs. baseline). Heart rate and mean arterial pressure were examined at 2, 4, 24, and 72 h after LPS treatment. In comparison to saline control, there was no significant change in these hemodynamic parameters at the time points examined. Only slightly lower mean arterial pressure was observed at 4 h after LPS treatment (82.1 ± 9.2 vs. 93.0 ± 6.5 mmHg in saline control, P > 0.05).

Time course of LPS-induced cardiac functional protection against ischemia. The baseline (before ischemia) coronary flow was 20.3 ± 0.56 ml/min and postischemic (at the end of reperfusion) coronary flow recovered to 16.9 ± 0.51 ml/min in the combined saline control group. The baseline coronary flow increased significantly at 12 h (23.8 ± 0.91 ml/min, P < 0.05 vs. saline control) and slightly at 24 h, 3 days, and 7 days after LPS treatment. Postischemic coronary flow was enhanced significantly at 12 h, 24 h, and 3 days (21.0 ± 0.71, 20.6 ± 0.68, and 20.5 ± 0.58 ml/min, respectively), and slightly at 7 days (19.8 ± 0.85 ml/min) (Fig. 1A). In contrast, LPS treatment 2 h before heart isolation did not affect either baseline or postischemic coronary flow. The baseline LVDP was depressed at 12 h after LPS treatment. At 2 h, 24 h, 3 days, and 7 days after LPS treatment, the baseline LVDP was compatible with that of the saline control hearts. After ischemia-reperfusion, LVDP recovered to 47.1 ± 2.9 mmHg in saline control hearts. Treatment with LPS 24 h, 3 days, or 7 days earlier significantly improved the postischemic recovery of LVDP. The maximal protection appeared at 3 days after LPS treatment (80.4 ± 3.1 mmHg, Fig. 1B). However, postischemic LVDP was not improved at 2 or 12 h after LPS treatment. Treatment with LPS 24 h, 3 days, or 7 days earlier also significantly attenuated postischemic contracture in compari-

Fig. 1. Time course of lipopolysaccharide (LPS)-induced cardiac functional protection against ischemia. Rats were treated with LPS (0.5 mg/kg ip) or normal saline (0.4 ml ip). Hearts were isolated and subjected to global ischemia-reperfusion at 2, 12, or 24 h, 3 days, or 7 days after the treatment. Coronary flow (A) and left ventricular developed pressure (LVDP; B) were assessed before ischemia and at end of reperfusion. Postischemic left ventricular end-diastolic pressure (LVEDP; C) was assessed at end of reperfusion. Data are means ± SE; n, no. of hearts. *P < 0.05 vs. saline control.
son to saline control (Fig. 1C). Similarly, cardiac protection against postischemic contracture was not observed at 2 or 12 h after LPS treatment.

Cardiodynamics during ischemia-reperfusion in maximally protected hearts and effects of cycloheximide pretreatment on maximal protection. Because LPS-induced cardiac functional protection was maximal at 3 days, detailed cardiodynamics during ischemia-reperfusion and effects of cycloheximide pretreatment on cardiac protection were examined at this time point. Three days after LPS treatment with or without cycloheximide pretreatment, baseline cardiac function was not different from saline control. As presented in Fig. 2, normothermic global ischemia resulted in a rapid decline in LVDP and a progressive increase in LVEDP. In all hearts examined, LVDP declined to an undetectable level within 5 min of global ischemia and remained at that level through the remainder of the ischemic period. Heart arrest was also observed in all other LPS-treated groups (data not shown). Similarly, LVEDP increased in all hearts examined during ischemia. LVEDP of LPS-treated hearts reached a peak level earlier than that of control hearts, whereas peak ischemic contracture did not differ between the two groups. LPS treatment 3 days before heart isolation improved postischemic recovery of cardiac function during reperfusion. This group of hearts exhibited improved postischemic LVDP relative to control hearts at all time points examined (Fig. 2A). Compared with the control hearts, hearts isolated 3 days after LPS treatment also demonstrated improved LVEDP recovery during reperfusion (Fig. 2B). The beneficial effects of LPS on postischemic functional recovery were abolished by cycloheximide pretreatment alone, although cycloheximide treatment alone did not affect postischemic functional recovery (Fig. 2).

Induction of myocardial and circulating TNF-α by LPS and inhibition by dexamethasone. A low level of TNF-α (0.28 ± 0.05 ng/g wet tissue) was detected in the myocardium of saline-treated rats, whereas TNF-α was undetectable in the serum. After administration of the sublethal dose of LPS, myocardial and serum TNF-α increased primarily at 1 and 2 h (Fig. 3). Myocardial TNF-α level increased by 5.6-fold at 1 h. The peak serum TNF-α level (9.1 ± 0.92 ng/ml, P < 0.05 vs. saline control) was also observed at 1 h. By 4 h after LPS treatment, TNF-α, both in serum and in myocardium, had returned to levels similar to the baseline. As shown in Fig. 4, dexamethasone at both doses applied abolished the increase in myocardial TNF-α level at 1 h after administration of LPS. Dexamethasone also dose-dependently attenuated the increase in myocardial TNF-α level at 1 h after administration of LPS (4.11 ± 0.84 ng/ml in dexamethasone 4.0 mg/kg + LPS group, P < 0.05 vs. LPS alone and P < 0.05 vs. saline control; 1.27 ± 0.66 ng/ml in dexamethasone 8.0 mg/kg + LPS group, P < 0.05 vs. LPS alone).

Effects of dexamethasone pretreatment on LPS-induced cardiac protection. Pretreatment of rats with dexamethasone at either dose applied (4.0 or 8.0 mg/kg ip) did not affect the beneficial effects of LPS on postischemic coronary flow and contractile function observed at 3 days after LPS treatment. After ischemia-reperfusion, coronary flow recovered to 20.2 ± 0.75 and 19.3 ± 0.54 ml/min, respectively, in dexamethasone + LPS groups (P < 0.05 vs. saline control; Fig. 5A). LVDP recovered to 78.5 ± 7.0 and 73.4 ± 4.3 mmHg, respectively (P < 0.05 vs. saline control; Fig. 5B). LVEDP recovered to 29.7 ± 3.6 and 33.7 ± 3.0 mmHg, respectively (P < 0.05 vs. saline control; Fig. 5C). These values were not different from those of the LPS 3-day group. However, dexamethasone alone administered 3 days before heart isolation did not improve postis-
Effects of TNFBP pretreatment on LPS-induced cardiac protection.

In the preliminary experiments, we observed that TNFBP at both doses used in this study abolished LPS-induced cardiac contractile depression (not shown). However, pretreatment of rats with TNFBP at either dose applied (40 or 80 µg/kg iv) did not affect the beneficial effects of LPS on postischemic coronary flow and contractile function. After ischemia-reperfusion, coronary flow recovered to 18.9 ± 0.66 and 19.1 ± 0.88 ml/min, respectively, in TNFBP + LPS groups (P < 0.05 vs. saline control; Fig. 6A). LVDP in TNFBP + LPS groups recovered to 72.0 ± 6.3 and 75.6 ± 9.6 mmHg, respectively (P < 0.05 vs. saline control; Fig. 6B), and LVEDP recovered to 23.0 ± 4.0 and 26.8 ± 5.5 mmHg, respectively (P < 0.05 vs. saline control; Fig. 5C). All of these function parameters in TNFBP + LPS groups were compatible to those of the LPS group. Similarly, TNFBP alone administered 3 days before heart isolation did not improve postischemic coronary flow and contractile function in comparison to saline control (Fig. 5).

**DISCUSSION**

We previously reported that a sublethal dose of LPS (0.5 mg/kg ip) depresses cardiac contractility in rats at 4–12 h after treatment (24) and that cardiac resistance to postischemic dysfunction is enhanced at 24 h after a treatment with this sublethal dose of LPS (4). The results of the present study show that the enhancement of cardiac resistance to ischemia required >12 h to develop and manifested at 24 h after LPS treatment, coincident with the recovery of the heart from endotoxemic depression (24). Cardiac resistance to ischemia was maximal at 3 days and persisted to 7 days after LPS treatment. The maximal cardiac protection induced by LPS was abolished by cycloheximide pretreatment.
ment, suggesting the involvement of de novo protein synthesis in the acquisition of the enhanced cardiac resistance to ischemia. Indeed, LPS induced myocardial and circulating TNF-α, which preceded the acquisition of the enhanced cardiac resistance to ischemia. However, the antecedent increase in myocardial and circulating TNF-α appears not to be required for LPS-induced cardiac resistance to ischemia, because dexamethasone pretreatment did not affect LPS-induced cardiac functional protection against ischemia, even though it abolished the increase in myocardial TNF-α and substantially suppressed circulating TNF-α. Furthermore, TNFBP given immediately after LPS treatment also failed to abrogate or attenuate LPS-induced cardiac functional protection against ischemia.

Tolerance to LPS toxicity develops after repeated administration of small doses of LPS to animals and is characterized by an attenuated response to a subsequent challenge with a large dose of homologous or heterologous LPS. Previous studies demonstrated that animals rendered tolerant are refractory to the lethal, metabolic, and pyrogenic effects of LPS (see Ref. 9). We have observed that a single exposure of rats to a small dose of LPS (0.5 mg/kg) 24 h earlier induces cardiac resistance to the cardiac depressive effect of the same dose of LPS (24) and that rat heart is also resistant to postischemic dysfunction at 24 h after a single treatment with this dose of LPS (4). It is likely that LPS pretreatment induces a cardiac cross-resistant state. It is not clear, however, when cardiac resistance to ischemia is acquired and whether LPS-induced cardiac functional protection against ischemia is transient or sustained. In this study, we examined the time course of LPS-induced cardiac functional protection against ischemia. Hearts did not acquire resistance to ischemia at 2 or 12 h after LPS treatment. At 2 h, hearts have not undergone endotoxemic myocardial depression, and at 12 h, hearts have not fully recovered from the depression (22, 24). Hearts acquired resistance to ischemia at 24 h, which is coincident with the recovery from endotoxemic myocardial depression. The resistance was maximal at 3 days and persisted to 7 days after LPS treatment. Clearly, rat hearts developed delayed and sustained resistance to ischemia after LPS treatment.

Myocardial ischemia impairs both systolic and diastolic properties in the heart, as evidenced by decreased LVDP and increased LVEDP during ischemia-reperfusion. LPS treatment did not prevent either the loss of contractility or ischemic contracture during ischemia, i.e., similar heart arrest and ischemic contracture in all LPS-treated hearts in comparison to controls. However,
chemic LVEDP. Ischemia-reperfusion also decreases coronary flow. The parallel changes in coronary flow and myocardial contractility suggest a possible relation between these two parameters. Indeed, coronary hypoperfusion itself can lead to myocardial dysfunction. Conversely, cardiac dysfunction may result in decreased coronary flow. In the Langendorff preparation, hearts were perfused at a constant pressure. Thus coronary flow is determined by intrinsic vascular resistance. The reduced coronary flow during reperfusion may reflect the decreased postischemic cardiac compliance. Thus improved postischemic coronary flow in the ischemia-resistant hearts may be, at least partly, the result of attenuated postischemic contracture. LPS treatment significantly increased baseline coronary flow at 12 h after treatment. Moreover, baseline coronary flow tended to be higher at 24 h to 7 days after LPS treatment. A number of studies have shown that LPS induces the expression of iNOS and increases nitric oxide (NO) production in the rat heart (17, 29). It is likely that the elevated baseline coronary flow is caused by an increased NOS activity in LPS-treated heart. Postischemic coronary flow was elevated in hearts isolated from rats treated with LPS 12 h to 7 days earlier. However, improved postischemic LVDP was observed only at 24 h to 7 days after LPS treatment. Postischemic coronary flow was dissociated from postischemic LVDP in hearts isolated from rats treated with LPS 12 h earlier. It is likely that elevated postischemic coronary flow may not directly contribute to the improved postischemic contractile function.

Hemorrhagic shock (21) can enhance cardiac resistance to ischemia. However, our previous work (22, 24) and the present study indicate that LPS at this low dose does not cause shock but induces myocardial depression in the rat. LPS-induced cardiac functional protection against ischemia-reperfusion injury may be a nonspecific protective response similar to those observed after hyperthermia (6, 13, 15, 18), ischemia (18), hypoxia (30), hemodynamic stress (23), or restraint stress (20). A time lag of >12 h suggests that the acquisition of LPS-induced cardiac functional protection against ischemia may involve de novo protein synthesis and accumulation of protective proteins. Indeed, LPS-induced cardiac functional protection against ischemia can be abolished by cycloheximide pretreatment. It is not clear from this study whether protein synthesis is involved in the signaling to the protective mechanisms, directly contributes to the enhanced cardiac resistance to ischemia, or both. The proteins involved in the protection remain unknown. LPS induces heat shock protein 72 in the myocardium (24) and

![Graph A](null)  
**Coronary Flow (ml/min)**  
- Preischemia  
- Post-reperfusion  
- Saline  
- TNFBP 80 µg/kg  
- LPS  
- TNFBP 40 µg/kg  
- LPS  
- TNFBP 80 µg/kg + LPS  
- LPS  
- *P < 0.05 vs. saline control.

![Graph B](null)  
**LVDP (mmHg)**  
- Preischemia  
- Post-reperfusion  
- Saline  
- TNFBP 80 µg/kg  
- LPS  
- TNFBP 40 µg/kg + LPS  
- LPS  
- TNFBP 80 µg/kg + LPS  
- LPS  
- *P < 0.05 vs. saline control.

![Graph C](null)  
**LVEDP (mmHg)**  
- Preischemia  
- Post-reperfusion  
- Saline  
- TNFBP 80 µg/kg  
- LPS  
- TNFBP 40 µg/kg + LPS  
- LPS  
- TNFBP 80 µg/kg + LPS  
- LPS  
- *P < 0.05 vs. saline control.

Fig. 6. Effects of TNF binding protein (TNFBP) on LPS-induced cardiac functional protection against ischemia. Rats were treated with TNFBP (40 or 80 µg/kg iv) immediately after LPS treatment (0.5 mg/kg ip). Hearts were isolated and subjected to global ischemia-reperfusion at 3 days after LPS treatment. Coronary flow (A) and LVDP (B) were measured during equilibration and at end of reperfusion. Postischemic LVEDP (C) was measured at end of reperfusion. Data are means ± SE; n = 3 in group treated with TNFBP alone, n = 5 in TNFBP + LPS groups. *P < 0.05 vs. saline control.
increases myocardial antioxidant enzyme activity (4, 19), which may be the result of de novo synthesis of enzymes or the modification of preexisting proteins. However, the roles of heat shock protein 72 and catalase in LPS-induced cardiac functional protection against ischemia remain to be determined.

LPS also promotes the expression of iNOS in the myocardium, increasing local NO production (17, 29). Although NO may not mediate the myocardial depression in this rat endotoxemia model (22), it may contribute to the elevated baseline coronary flow. It is clear that NO plays an important role in intracellular signal transduction, and perhaps NO plays a role in transducing the LPS signal leading to myocardial adaptation to ischemia. Similarly, the potential role of NO in LPS-induced functional protection against ischemia remains to be determined.

LPS induces the synthesis of proinflammatory cytokine TNF-α by circulating monocytes, tissue-resident macrophages, and cardiac myocytes (8, 10, 11, 33). Exogenous TNF-α can induce general resistance to LPS (9) and cardiac resistance to ischemia-reperfusion injury (3, 7, 25). Thus endogenous TNF-α may play important roles, as a mediator, in the induction of cardiac protective response by LPS. The present study focused on the question of whether endogenous myocardial and circulating TNF-α is required for the induction of cardiac protection by LPS. After LPS treatment, myocardial and circulating TNF-α levels increased at 1 and 2 h and normalized at 4 h. It is impossible for myocardial TNF-α to directly contribute to the enhanced cardiac resistance to ischemia, because the enhanced cardiac resistance to ischemia developed after myocardial TNF-α normalized. LPS-induced myocardial and circulating TNF-α may serve as a mediator in the induction of the cardiac protective response by LPS, as suggested by previous studies (3, 7, 25). However, our present data do not support this hypothesis. Although the cardioprotective dose of LPS induced an increase in circulating TNF-α level (9.1 ng/ml), it may be far below the circulating TNF-α level necessary for the induction of cardiac protection against ischemia. A large dose of TNF-α (30 µg/kg or 10 µg/rat for rats of 250–300 g) used in previous studies may result in a huge increase in circulating TNF-α level (~570–770 ng/ml blood by calculation). Indeed, LPS-induced increase in myocardial and circulating TNF-α preceded the acquisition of the enhanced cardiac resistance to ischemia. However, the abolishment of the increase in myocardial TNF-α and suppression of circulating TNF-α with dexamethasone pretreatment did not affect LPS-induced cardiac protection against ischemia. Moreover, LPS-induced cardiac functional protection against ischemia remained intact in rats treated with TNF-αB immediately after LPS treatment. Thus it appears that LPS induces delayed cardiac functional protection against ischemia independent of LPS-induced increase in myocardial and circulating TNF-α. The expression of TNF-α and the enhancement of cardiac resistance to ischemia may be two unrelated responses to LPS.

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