Lipoteichoic Acid Induces Delayed Protection in the Rat Heart
A Comparison With Endotoxin

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Abstract—Classic ischemic preconditioning transiently (30 to 120 minutes) protects the myocardium against subsequent lethal ischemia/reperfusion injury. After dissipation of this acute protection, a second window of protection (SWOP) appears 12 to 24 hours later; this SWOP lasts up to 3 days. Several triggers induce a SWOP, including brief repetitive cycles of coronary artery occlusion, rapid ventricular pacing, stimulation of adenosine A1 receptors, and administration of wall fragments of Gram-negative bacteria, such as lipopolysaccharide (LPS). The aim of this study was to investigate whether lipoteichoic acid (LTA), a cell wall fragment of Gram-positive bacteria, can induce a SWOP in a rat model of left anterior descending coronary artery (LAD) occlusion (25 minutes) and reperfusion (2 hours). Thus, 166 male Wistar rats were pretreated (2 to 24 hours) with saline, LTA (1 mg/kg IP), or LPS (1 mg/kg IP) and subjected to LAD occlusion/reperfusion. Pretreatment with LTA or LPS for 16 hours led to a substantial, \( \approx 65\% \), reduction in infarct size and a reduction in the release of cardiac troponin T into the plasma. The dose of LTA used had no toxic effect (on any of the parameters studied), whereas the same dose of LPS caused a time-dependent activation of the coagulation system and liver injury. By use of RNase protection assays, it was determined that LPS caused a time-dependent induction of tumor necrosis factor-\( \alpha \), interleukin-1\( \beta \), and manganese superoxide dismutase mRNA content in the heart, whereas LTA failed to induce manganese superoxide dismutase. LPS also caused an upregulation of the expression of intercellular adhesion molecule-1 and P-selectin, whereas LTA downregulated these molecules and attenuated the accumulation of polymorphonuclear granulocytes caused by myocardial ischemia/reperfusion. This study demonstrates for the first time that pretreatment with LTA at 8 to 24 hours before myocardial ischemia significantly reduces (1) infarct size, (2) cardiac troponin T, and (3) the histological signs of tissue injury in rats subjected to LAD occlusion and reperfusion. The mechanism(s) underlying the observed cardioprotective effects of LTA warrants further investigation but is likely to be related to its ability to inhibit the interactions between the coronary vascular endothelium and polymorphonuclear granulocytes. Therefore, LTA represents a novel and promising agent capable of enhancing myocardial tolerance to ischemia/reperfusion injury. (Arterioscler Thromb Vasc Biol. 2000;20:1521-1528.)

Key Words: lipopolysaccharide \( \bullet \) lipoteichoic acid \( \bullet \) myocardial infarct size \( \bullet \) delayed preconditioning \( \bullet \) myocardial ischemia

During coronary artery angioplasty or open heart surgery, regional or global iatrogenic cardiac ischemia is a common event. Reperfusion of previously ischemic myocardium leads to ischemia/reperfusion injury with enhanced propagation of arrhythmias,1 stunning,2 and cardiomyocyte cell death.3 Over the last few years, the population of patients with coronary artery disease undergoing coronary artery angioplasty or bypass surgery has increased, as have associated complications, eg, myocardial infarction4 and myocardial stunning.2 Thus, there is a considerable interest in the development of new strategies to reduce ischemia/reperfusion injury.

Classic ischemic preconditioning, eg, brief periods of coronary artery occlusion and reperfusion, transiently (30 to 120 minutes) protects the myocardium against subsequent lethal ischemia/reperfusion injury and is mediated by the activation of G protein–coupled membrane receptors and intracellular downstream kinases.5–7 After dissipation of this acute protection, a second window of protection (SWOP) appears 12 to 24 hours later, which lasts up to 3 days and requires the synthesis of new proteins.8,9 In addition to reducing myocardial infarct size,8,10,11 the SWOP enhances the resistance of the myocardium against further arrhythmias,12 posts ischemic endothelial dysfunction,13 and myocardial stunning.14
In the heart, several triggers induce a SWOP, including brief repetitive cycles of coronary artery occlusion,\textsuperscript{8} ventricular rapid pacing,\textsuperscript{15} stimulation of adenosine A\textsubscript{1} receptors,\textsuperscript{10} and wall fragments of Gram-negative bacteria, such as lipopolysaccharide (LPS or endotoxin)\textsuperscript{11,15} or the structurally related monophosphoryl lipid A (MLA).\textsuperscript{16} LPS and MLA contain lipid A, and this moiety mediates many of the biological effects of LPS and MLA, including the induction of catalase,\textsuperscript{15} manganese superoxide dismutase (Mn-SOD),\textsuperscript{17,18} heat shock proteins,\textsuperscript{19} tumor necrosis factor (TNF)-α,\textsuperscript{16} interleukin (IL)-1β,\textsuperscript{20} P-selectin,\textsuperscript{21} intercellular adhesion molecule (ICAM)-1,\textsuperscript{21} and activation of polymorphonuclear granulocytes (PMNs).\textsuperscript{22} Although MLA is less toxic than LPS, it is still a potent immunomodulator, which induces the release of cytokines and the expression of adhesion molecules. Recently, Elliott et al.\textsuperscript{23} have presented a new second-generation synthetic glycolipid, RC-552, which induces SWOP without immunostimulatory effects.

However, since the formal recognition of the SWOP,\textsuperscript{8} several studies have investigated the underlying mechanism of the ability of the heart to adapt to stressful stimuli. To date, 3 different protein families, namely, antioxidant enzymes such as Mn-SOD,\textsuperscript{24} heat shock proteins such as HSP 70,\textsuperscript{25} and inducible NO synthase (iNOS),\textsuperscript{26} have received the most attention as possible mediators/effectors of the delayed protection of the heart. More recently, Guo et al.\textsuperscript{27} have extended their work to examine the possible role of iNOS in mediating delayed preconditioning against infarction. On the basis of the pharmacological concept that delayed preconditioning was abrogated by nonselective NO synthase inhibitors\textsuperscript{28,29} and because the administration of NO donors in the absence of ischemia was found to induce delayed protection,\textsuperscript{30} Guo et al. have used the availability of iNOS gene knockout mice to demonstrate that targeted ablation of the iNOS gene abrogates delayed preconditioning of mice hearts. Although the upregulation of the iNOS gene was modest after ischemic preconditioning compared with inflammation or septic shock, these results support the view that an enhanced formation of NO by iNOS contributes to the cardioprotective effects of delayed preconditioning.

Gram-positive organisms, however, do not contain LPS, which is the cell wall component of Gram-negative bacteria responsible for the initiation of Gram-negative septic shock. Gram-positive bacteria can also cause septic shock and multiple organ failure without causing endotoxemia,\textsuperscript{31} and endotoxin is not always found in the serum of patients with septic shock.\textsuperscript{32} The cell wall of Gram-positive bacteria contains lipoteichoic acid (LTA) and peptidoglycan (PepG). LTA is a macroamphiophile, equivalent to LPS in Gram-negative bacteria, containing a substituted polyglycerophosphate backbone attached to a glycolipid.\textsuperscript{33} PepG is a large polymer, which provides stress resistance and shape-determining properties to bacterial cell walls.

Currently, it is not known whether LTA can induce a SWOP in the heart. Therefore, the aim of the present study was to investigate whether LTA triggers a SWOP in a rat model of regional myocardial ischemia and reperfusion. Having found that pretreatment of rats with LTA protects the heart against subsequent ischemia/reperfusion injury, we have (1) compared the SWOP caused by LTA with the SWOP caused by LPS and (2) attempted to investigate the putative mechanism(s) by which LTA causes a SWOP.

### Methods

One hundred sixty-six male Wistar rats (220 to 280 g) were obtained from Tuck (Rayleigh, Essex, UK) and cared for according to American Association for Accreditation of Laboratory Animal Care guidelines and the Guide for the Care and Use of Laboratory Animals (Department of Health and Human Services, National Institutes of Health, publication No. 86-23).

#### Myocardial Ischemia and Reperfusion in the Rat In Vivo

The technique used to produce left anterior descending coronary artery (LAD) occlusion was identical to that described previously.\textsuperscript{11} Briefly, rats were anesthetized with thiopental sodium (120 mg/kg IP). The trachea was cannulated, and artificial respiration was maintained by use of a Harvard ventilator with a frequency of 70 strokes per minute, a tidal volume of 8 to 10 mL/kg, an inspiratory oxygen concentration of 30%, and a positive end-expiratory pressure of 1 to 2 mm Hg, resulting in P\textsubscript{CO}\textsubscript{2} values of 36 to 44 mm Hg and P\textsubscript{O}\textsubscript{2} values >150 mm Hg. Body temperature was maintained at 38.0° to 39.0°C. The right carotid artery was cannulated to monitor mean arterial blood pressure (MAP) and heart rate (HR). The pressure rate index (PRI), a relative indicator of myocardial oxygen consumption, was calculated as the product of MAP and HR (expressed as mm Hg/(min×10\textsuperscript{3})). The right jugular vein was cannulated for administration of drugs. The chest was opened by a left-side thoracotomy, the pericardium was incised, and an atrumatic needle and occluder were placed around the LAD. After completion of the surgical procedure, the animals were allowed to stabilize for 30 minutes before LAD ligation, which allowed constriction of the occluder (time 0). This was associated with the typical hemodynamic changes observed during myocardial ischemia, eg, a fall in MAP. After 25 minutes of acute myocardial ischemia, the occluder was released, allowing the reperfusion of the previously ischemic myocardium for 2 hours. After reoccluding the LAD, Evans blue dye (1 mL of 2% [wt/vol]) was administered intravenously to determine ischemic myocardium (area at risk [AR]) and nonischemic myocardium (area not at risk). Subsequently, the heart was cut into horizontal slices and then into small pieces. The AR was separated from the area not at risk and then incubated with p-nitro blue tetrazolium (0.5 mg/mL, 20 minutes at 37°C) to distinguish between ischemic and infarcted tissue;\textsuperscript{34} the area not at risk was incubated with saline. The AR and infarct size were calculated after weighing the respective tissue samples and expressed as percentage of the AR.

Rats were randomly allocated into the following groups, which were injected intraperitoneally and subjected to either no occlusion of the LAD (sham) or 2-hour reperfusion (I-R): group 1, saline (1 mL/kg, n=6) at 16 hours, sham; group 2, saline (1 mL/kg, n=10) at 16 hours, I-R; group 3, LPS (1 mg/kg, n=6) at 16 hours, sham; group 4, LPS (1 mg/kg, n=5) at 2 hours, I-R; group 5, LPS (1 mg/kg, n=5) at 4 hours, I-R; group 6, LPS (1 mg/kg, n=8) at 8 hours, I-R; group 7, LPS (1 mg/kg, n=8) at 16 hours, I-R; group 8, LPS (1 mg/kg, n=7) at 24 hours, I-R; group 9, LTA (1 mg/kg, n=6) at 16 hours, sham; group 10, LTA (1 mg/kg, n=6) at 2 hours, I-R; group 11, LTA (1 mg/kg, n=6) at 4 hours, I-R; group 12, LTA (1 mg/kg, n=5) at 8 hours, I-R; group 13, LTA (1 mg/kg, n=8) at 16 hours, I-R; and group 14, LTA (1 mg/kg, n=6) at 24 hours, I-R. The letter n refers to the number of rats that survived until the end of the experiment. The numbers of rats that died in the individual groups were as follows: group 2, n=2; group 4, n=1; group 5, n=1; group 8, n=1; and group 12, n=1.

#### Measurements

The following methods were used as described in detail previously:

1. Plasma levels of cardiac troponin T (cTnT) were determined. At the end of the experiment, a blood sample was obtained and centrifuged to obtain plasma. The plasma supernatants were injected intraperitoneally and subjected to either no occlusion of the LAD (sham) or 2-hour reperfusion (I-R): group 1, saline (1 mL/kg, n=6) at 16 hours, sham; group 2, saline (1 mL/kg, n=10) at 16 hours, I-R; group 3, LPS (1 mg/kg, n=6) at 16 hours, sham; group 4, LPS (1 mg/kg, n=5) at 2 hours, I-R; group 5, LPS (1 mg/kg, n=5) at 4 hours, I-R; group 6, LPS (1 mg/kg, n=8) at 8 hours, I-R; group 7, LPS (1 mg/kg, n=8) at 16 hours, I-R; group 8, LPS (1 mg/kg, n=7) at 24 hours, I-R; group 9, LTA (1 mg/kg, n=6) at 16 hours, sham; group 10, LTA (1 mg/kg, n=6) at 2 hours, I-R; group 11, LTA (1 mg/kg, n=6) at 4 hours, I-R; group 12, LTA (1 mg/kg, n=5) at 8 hours, I-R; group 13, LTA (1 mg/kg, n=8) at 16 hours, I-R; and group 14, LTA (1 mg/kg, n=6) at 24 hours, I-R. The letter n refers to the number of rats that survived until the end of the experiment. The numbers of rats that died in the individual groups were as follows: group 2, n=2; group 4, n=1; group 5, n=1; group 8, n=1; and group 12, n=1.
Measurement of Plasma Levels of TAT in the Rat

The conversion of prothrombin into active thrombin is a key event within the coagulation cascade. Thrombin acts on various physiological substrates, eg, protein C or platelets, and is inhibited by antithrombin III. The inhibition complex (inactive proteinase/inhibitor complex) can be measured quantitatively by enzyme immunoassay.23 There is a significant correlation between elevated concentrations of the thrombin/antithrombin III complex (TAT) and thrombotic events, eg, in patients with septic shock.24 After pretreatment of the rats with saline, LPS, or LTA (2 to 24 hours, n = 3 or 4), a blood sample was centrifuged to obtain plasma, and the concentration of TAT was determined by using the Enzygnost TAT microimmunoassay (Behringwerke AG).

Probes

The rat Mn-SOD cDNA probe was cloned by polymerase chain reaction with the use of 5′-GTC GCT TAC AGA TGG CCT GC-3′ as a 5′ primer and 5′-CTA CTA CAA AAC ACC CAC GC-3′ as a 3′ primer.41 The cDNA fragment corresponds to nucleotides 481 to 731 of the published sequence.41 The cDNA fragment of rat IL-1β corresponds to nucleotides 960 to 1222 (unpublished sequence, EMBL Databank access No. M98820).

Figure 1. Myocardial ischemia caused by occlusion (25 minutes) and reperfusion (2 hours) of the LAD in the anesthetized rat. Infarct size is expressed as percentage of AR caused by occlusion and reperfusion of the LAD. Separate groups of animals were pretreated intraperitoneally with saline (con, n = 10), with LPS (1 mg/kg) at different time points before experimental intervention at 2 hours (n = 5), 4 hours (n = 5), 8 hours (n = 8), 16 hours (n = 8), or 24 hours (n = 7), or with LTA (1 mg/kg) at 2 hours (n = 8), 4 hours (n = 6), 8 hours (n = 5), 16 hours (n = 8), or 24 hours (n = 6). ∗P < 0.05 vs con.

EMBL Databank access No. M98820. The cDNA fragment of rat TNF-α corresponds to nucleotides 301 to 550 of the published sequence.44

Drugs and Materials

Unless otherwise stated, all compounds were obtained from Sigma Chemical Co. LPS was obtained from Escherichia coli serotype 0127:B8. Thiopental sodium (Intraval) was obtained from May & Baker Ltd.

Statistical Analysis

Data are reported as mean±SD for n observations. ANOVA with the Bonferroni test was used to compare groups. A value of P<0.05 was considered statistically significant.

Results

Cardioprotective Effects of LPS or LTA In Vivo

The mean values for AR were similar in all animal groups studied and ranged from 45% to 56% (P<0.05, data not shown). In rats pretreated with saline, occlusion of the LAD (for 25 minutes) followed by reperfusion (for 2 hours) resulted in an infarct size of 60% (n = 10) of the AR. Compared with vehicle, 1 mg/kg LPS administered at different time points before coronary artery ligation caused a time-dependent reduction in infarct size (from 56% at 2 hours to 23% at 24 hours, P<0.05; Figure 1). Compared with vehicle, 1 mg/kg LTA administered at different time points before coronary artery ligation also caused a time-dependent reduction in infarct size (from 56% at 2 hours to 25% at 24 hours, P<0.05; Figure 1). Sham operation alone, with no occlusion of the LAD (16-hour pretreatment with saline, LPS, or LTA; each n = 6), did not result in a significant degree of infarction in any of the animal groups studied (<3% of the AR, data not shown).

Emphysematous Effects of LPS or LTA During Myocardial Ischemia and Reperfusion

Emphysematous data, ie, MAP, HR, and PRI, measured during the course of the experiments, were similar in all groups studied (P>0.05, data not shown). In sham-operated rats (no LAD occlusion), injection of vehicle (saline), LPS, or LTA at 16 hours before surgery did not cause any significant effects.

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Histological evaluation by light microscopy of biopsies of control hearts subjected to regional ischemia and reperfusion and received saline, LPS, or LTA; the mean values for MAP and PRI fell throughout the experiment (P>0.05, data not shown).

Effects of LPS or LTA on Plasma Levels of cTnT in the Rat

Pretreatment of the rats with saline followed by ischemia/reperfusion of the LAD resulted in a significant increase in the plasma levels of cTnT (Figure 2). Compared with vehicle, LPS pretreatment at different time points before ischemia caused a time-dependent reduction in the release of cTnT (P<0.05, Figure 2). Compared with vehicle, LTA pretreatment at different time points before ischemia also caused a time-dependent reduction in the release of cTnT (P<0.05, Figure 2). Sham operation alone (16-hour pretreatment with saline, LPS, or LTA; each n=6) did not result in a significant increase in the plasma levels of cTnT (<1 ng/mL, data not shown).

Effects of LPS or LTA on Histological Signs of Tissue Injury in Myocardium of the Rat

Histological evaluation by light microscopy of biopsies of control hearts subjected to regional ischemia and reperfusion demonstrated the occurrence of complete coagulation necrosis (data not shown). The cytoplasm of myocytes was deeply eosinophilic and accompanied by a substantial accumulation of PMNs between the necrotic cardiomyocytes. In addition, nuclear structures were absent (data not shown). The described signs were also detectable in sections and were stained with fuchsion and Luxol fast blue (data not shown). Rats pretreated with either LPS or LTA at 16 hours before ischemia/reperfusion had a significantly smaller infarct size.

The infarcted tissue of these animals demonstrated the same typical signs of necrosis as described above, e.g., hyperemia of blood vessels, cytoplasmic eosinophilia of cardiomyocytes and extravasation of red blood cells, but less accumulation of PMNs into the noninfarcted border region of the risk zone (see Figure 6c). Histological data are not shown (each group n=3 or 4).

Effects of LPS or LTA on Liver Enzyme Release and Renal Function/Injury Parameters In Vivo

Compared with vehicle, LPS or LTA administered at different time points (2 to 24 hours) did not result in any significant alterations in the plasma levels of urea and creatinine, demonstrating normal renal function (data not shown). Compared with vehicle, LPS administration resulted in a significant rise in the plasma levels of AST and ALT (P<0.05) after 8 hours, demonstrating the development of liver injury. Treatment of rats with LTA did not result in any alteration of these enzymes at any time point (data not shown, each group n=3 or 4).

Effects of LPS or LTA on TAT In Vivo

Compared with vehicle, LPS administration resulted in a significant alteration in the plasma levels of TAT at 4 and 8 hours (P<0.05), indicating a substantial activation of the coagulation system in the blood (Figure 3). Compared with vehicle, LTA administration did not result in any significant rise in the plasma levels of TAT (Figure 3, each group n=3 or 4).

Effects of LPS or LTA on mRNA Induction of TNF-α and IL-1β In Vivo

Figure 4a shows a typical result from the RNase protection assay, demonstrating the induction of TNF-α and IL-1β mRNA expression by pretreatment with either LPS or LTA. The time-dependent LPS- or LTA-induced increase in these cytokines is shown schematically in Figures 4b and 4c. The administration of LPS in rats resulted in a time-dependent increase of TNF-α and IL-1β mRNA content in the heart, with a maximum observed at 2 and 4 hours (Figures 4b and 4c). Compared with pretreatment with LPS, the administration of LTA resulted in a lower, but still significant, increase in TNF-α mRNA content at 2 hours (Figure 4b). Compared with control, pretreatment with LTA resulted in a significant increase in IL-1β mRNA content at 2 hours (Figure 4c). Compared with pretreatment with LPS, LTA did not result in any significant alteration in the IL-1β mRNA content at any time point (Figure 4c). Figure 4 represents groups of 3 or 4.

Effects of LPS or LTA on mRNA Induction of Mn-SOD In Vivo

Figure 5a shows a typical result from the RNase protection assay, demonstrating the induction of Mn-SOD mRNA ex-
pression by pretreatment with either LPS or LTA. The time-dependent LPS- or LTA-induced increase in the mRNA content of Mn-SOD is shown schematically in Figure 5b. Administration of LPS to rats resulted in a time-dependent increase of Mn-SOD mRNA content in the heart, with a maximum observed at 4 and 8 hours, which normalized after 16 and 24 hours (Figure 5b). LTA pretreatment had no significant effect on the Mn-SOD mRNA content in the rat heart. Figure 6 represents groups of 3 or 4.

**Effects of LPS or LTA on P-Selectin/ICAM-1 Expression and Accumulation of PMNs In Vivo**

A substantial degree of P-selectin and ICAM-1 expression was observed in the postischemic endothelium from the hearts of rats that had been treated with saline and subjected to regional myocardial ischemia and reperfusion (Figures 6a and 6b). Administration of LPS 16 hours before ischemia/reperfusion resulted in an increased expression of P-selectin and ICAM-1. Surprisingly, LTA pretreatment caused a significant downregulation of P-selectin and ICAM-1 (Figures 6a and 6b). In addition, we have determined the PMN accumulation in histological slices of controls in the border zone of the infarcted tissue (Figure 6c). Compared with control, LPS as well as LTA pretreatment caused a significant reduction of PMN accumulation in the border zone. Figure 6 represents groups of 3 or 4.

**Discussion**

Classic ischemic preconditioning, eg, brief periods of coronary artery occlusion and reperfusion, transiently (30 to 120 minutes) protects the myocardium against subsequent lethal ischemia/reperfusion injury. After dissipation of this acute protection, a SWOP appears 12 to 24 hours later, which lasts up to 3 days. In the heart, several triggers induce a SWOP, including wall fragments of Gram-negative bacteria, such as LPS (endotoxin) or the structurally related MLA. The present study demonstrates for the first time that pretreatment (for 8 to 24 hours) with LTA, a cell wall fragment of Gram-positive bacteria, protects the heart against a subsequent period of regional ischemia and reperfusion. LTA reduces myocardial infarct size as well as the release of cTnT, and the magnitude of the cardioprotective effect of LTA (60% to 70% reduction in infarct size) was similar to the one produced by LPS at the same dose. Unlike LPS, however, LTA did not cause an activation of the coagulation cascade or liver injury. Thus, LTA (at 1 mg/kg IP) protects the heart against ischemic injury without causing significant side effects.
What, then, is the possible mechanism(s) by which LTA and LPS reduce the degree of necrosis caused by myocardial ischemia and reperfusion? Clearly, in both studies and in all groups studied, there were no significant differences in body weight, heart weight, AR, or hemodynamic parameters, such as MAP or HR, suggesting that the beneficial effects of LPS and/or LTA were not related to differences in the amount of myocardial tissue sampled or to the changes in myocardial oxygen demand.

It has been demonstrated that LPS potentiates inflammatory mediators such as TNF-α and IL-1β. TNF-α induces oxidant stress through the generation of reactive oxygen species and activates protein kinase and nuclear factor-κB, resulting in the expression of heat shock proteins or Mn-SOD or IL-1. The inflammatory cytokines TNF-α and IL-1β induce the expression of iNOS and, hence, generate NO. A potential role for the iNOS/NO system as a novel mediator/effector of delayed preconditioning of the heart has been described recently. However, in the present study, we have not investigated the role of iNOS/NO in the delayed protection caused by LPS or LTA. Although we have used a small dose of LPS or LTA in the present study, we have observed a cytokine response, which was greater in LPS-treated animals. The observed increase in the mRNA levels of the investigated cytokines TNF-α and IL-1β, if translated into protein, may induce iNOS and may finally cause cardioprotection. Nevertheless, this important issue warrants further investigation.

TNF-α also facilitates neutrophil migration across the vascular wall and activates PMNs, which generate reactive oxygen species and are generally considered to be the principal effectors of reperfusion injury. Within the mitochondrial matrix, Mn-SOD is an essential antioxidant enzyme that catalyzes the conversion of superoxide radical to hydrogen peroxide and molecular oxygen. Using a RNase protection assay, we have demonstrated that LPS pretreatment caused a significant induction of TNF-α and IL-1β mRNA at 2 to 4 hours. LTA pretreatment (compared with control) also caused a significant induction of the mRNAs of both cytokines. It should be noted that the induction of TNF-α or IL-1β mRNA by LTA was only 50% or 20% of the induction afforded by LPS, respectively. In addition, our results clearly demonstrate that LPS pretreatment caused a significant up-regulation of Mn-SOD mRNA at 4 to 8 hours. In contrast, LTA did not significantly upregulate Mn-SOD mRNA levels at 2 to 24 hours. However, in both cases (cytokines and Mn-SOD), we have determined the mRNA levels only. One could argue that an early peak of mRNA levels of these cytokines (at 2 hours) may translate into the respective proteins and, hence, may induce the Mn-SOD gene. Our data obtained from the LPS-treated groups suggest that there may have been a significant translation into the respective cytokines as Mn-SOD mRNA levels were significantly increased.
between 4 and 8 hours. Although not investigated in the present study, it is likely that the Mn-SOD gene is also translated into its respective protein. Therefore, one could expect increased Mn-SOD activity and cardioprotection after 4 to 8 hours, which may last up to 24 hours or longer (depending on the half-life of the enzyme).

One could argue that a 1.7-fold (2-hour value compared with control value) induction of Mn-SOD mRNA afforded by LTA could be cardioprotective. However, further investigations are warranted to confirm whether this small increase in mRNA also translates into a significant increase in protein and activity of Mn-SOD. One of the aims of the present study was to attempt to elucidate which genes are regulated as part of the cardioprotective mechanisms afforded by LPS or LTA. Although several candidate genes were not investigated, we measured changes in Mn-SOD mRNA levels and report in the present study for the first time that LTA pretreatment does not significantly increase the expression of Mn-SOD mRNA. Nevertheless, other mechanisms should also be investigated in future studies.

Reperfusion of previously ischemic tissue shares many characteristics with other inflammatory responses of the myocardium, including the generation of reactive oxygen species, cytokines, and other proinflammatory mediators, which activate PMNs and the coronary vascular endothelium. Numerous studies suggest that the activation of PMNs plays an important role in determining ultimate infarct size in models of ischemia followed by prolonged reperfusion. In the present study, we have demonstrated that pretreatment of rats with LPS for 16 hours results in an upregulation of the adhesion molecules P-selectin and ICAM-1. Surprisingly, pretreatment of rats with LTA caused a downregulation of these adhesion molecules and abolished the accumulation of PMNs in the border zone of the infarcted myocardium. Our results are in part confirmed by the findings of Lavkam et al., who demonstrated that LTA has no effects on PMN aggregation and adherence, whereas LPS significantly enhances the adherence and aggregation.

In conclusion, the present study demonstrates for the first time that pretreatment of rats with LTA or LPS for 8 to 24 hours significantly reduces (1) infarct size, (2) cTnT release, and (3) histological signs of tissue injury in rats subjected to myocardial ischemia/reperfusion. In addition, we have demonstrated that LTA at a dose of 1 mg/kg IP does not show studied signs of toxicity in rats. The mechanism(s) underlying the observed cardioprotective effects of LTA warrants further investigation but is likely to be related to its ability to inhibit interactions between the coronary vascular endothelium and PMNs. We propose that LTA represents a novel and promising agent capable of enhancing myocardial tolerance to ischemia/reperfusion injury.

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References

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