Research

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Effects of radiodetoxified endotoxin on nitric oxide production in J774 macrophages and in endotoxin shock

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Summary Radiodetoxified lipopolysaccharide (RD-LPS) is a ^{60}Co-gamma-irradiated LPS with a modified structure, which decreases its toxic effects. To obtain a better understanding of the mechanism of the reduced toxicity of RD-LPS, here we studied the effect of RD-LPS on the regulation of nitric oxide (NO) production in vitro and in vivo. In control cells, stimulation by native LPS (10 μg/ml) induced the expression of the inducible NO synthase (iNOS) and production of NO, as measured by increase in the concentration of nitrite, breakdown product of NO. Pre-exposure of the cells for 24 h to a subthreshold concentration of RD-LPS (10 ng/ml) induced a complete desensitization to the LPS-induced NO production in comparison to control cells ($P < 0.01$). On the contrary, pre-exposure of the cells with native LPS (10 ng/ml) did not reduce LPS-induced NO synthesis. RD-LPS induced a smaller production of tumor necrosis factor (TNF) than native LPS, but did not induce a desensitization against subsequent LPS-induced TNF synthesis. In in vivo studies, pretreatment of rats with repeated doses of sublethal RD-LPS (1 mg/kg/day i.p. for 4 days) inhibited increase of plasma nitrate/nitrite levels, NO production in peritoneal macrophages ex vivo and induction of lung iNOS activity, in response to a high-dose LPS challenge (15 mg/kg i.p.) given at the fifth day. Pretreatment with repeated sublethal doses of the native LPS (1 mg/kg/day i.p.) did not affect NO production in rats subjected to endotoxic shock. The results demonstrate that RD-LPS induces tolerance to the stimulatory effect of LPS on NO synthesis. Suppression of iNOS induction was only observed with RD-LPS, but not with native LPS in the models used herein. It remains to be further investigated whether suppression of iNOS induction by RD-LPS contributes to the protective effects of this compound in shock and inflammation.

INTRODUCTION

The lipopolysaccharide (LPS) component of the cell wall of Gram-negative bacteria is known as a major trigger of hypotension, multiple organ failure and death during septic shock.[1] Most of the biological effects elicited by endotoxin are, in part, mediated by the activation of macrophages and the subsequent release of proinflammatory mediators. Among them, nitric oxide (NO) has been demonstrated to contribute importantly to the hemodynamic and metabolic response during infection.[2] LPS and pro-inflammatory cytokines induce the production of NO from L-arginine by the inducible isoform of NO-synthase (iNOS) in a variety of cell types, including macrophages and in various tissues and organs such as spleen, lung, liver, heart and blood vessels.[3] An enhanced formation of NO acts as a cytostatic and cytotoxic molecule against fungal, bacterial, helminthic and protozoal organisms[3] and also contributes to the regulation of specific lymphocyte functions.[4] Overproduction of NO by NOS,

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however, also plays a crucial role in the pathophysiologic manifestation of peripheral vascular failure associated with septic shock.[2,5]

The induction of tolerance to systemic effects of endotoxin has been proposed as a potential form of immunomodulatory therapy. Prior exposure to small sublethal concentrations of LPS renders humans and laboratory animals resistant to otherwise lethal effects of LPS.[6,7] This acquired state of hyporesponsiveness has been attributed to reduction of the synthesis of proinflammatory mediators like TNF-α, IL-1, IL-6, arachidonic acid metabolites[8-10] and increased production of antiinflammatory mediators like IL-10 and transforming growth factor β.[11]

Endotoxin toxicity can be also abolished by detoxifying procedures.[12] Recently, a radiodetoxified endotoxin (RD-LPS) obtained by ^{60}Co-γ-irradiation, has been shown to elicit non-specific tolerance both in vivo and in vitro studies.[12-17] RD-LPS has been found to have reduced lethal and hemodynamic effects,[13] to prevent leukocyte infiltration in lungs[12] and hepatic[14] and pancreatic[15] damage after challenge with endotoxin, while it retains its capacity to increase the natural immune resistance.[16]

The aim of present experiments was, therefore, to compare the effect of pre-exposure to RD-LPS or its native form on NO synthesis in LPS-stimulated J774 macrophages and in in vivo experiments in rats subjected to endotoxic shock. Our results demonstrate that RD-LPS induces tolerance to the stimulatory effect by LPS of NO synthesis in vitro and in vivo.

MATERIALS AND METHODS

Cell culture

The murine macrophage line J774 was cultured in Dulbecco's modified Eagle's Medium (DMEM) containing 10% fetal calf serum in 96-well plates (90–100% confluence in 0.2 ml final volume) under standard conditions. Cells were pretreated for 24 h with different concentrations (1 ng to 10 μg/ml) of *Escherichia coli* 089 lipopolysaccharide (LPS) and its radiodetoxified form (RD-LPS) in order to determine an optimal subthreshold concentration. After the pretreatment period, the cells were washed twice with fresh medium and treated for a further 24 h period with a higher concentration of LPS (10 μg/ml), that is sufficient to stimulate NO production in J774 cells.[18] For the second treatment regimen, the same serotype of LPS (*E. coli* 089) was used, which was also used during the pretreatment protocols. Supernatant was collected for the measurement of nitrite and of tumor necrosis factor alpha (TNF) after the first 24 h incubation, as well as after the second 24 h stimulation period.

Cell viability

Mitochondrial respiration, an indicator of cell viability, was assessed using the mitochondrial dependent reduction of 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) to formazan.[18] At the end of each experiment, cells in 96-well plates were incubated with MTT (0.2 mg/ml) for 1 h at 37°C. The culture medium was removed and the cells were solubilized in dimethylsulphoxide (0.1 ml). The extent of reduction of MTT to formazan within cells was quantitated by measurement of OD_{550} by using the Spectramax microplate reader.

TNF production

TNF production in cell culture supernatants was determined by an ELISA kit specific against murine TNF (Genzyme Corp., Boston, MA, USA). Assays were performed according to the manufacturer's instructions and the colorimetric results quantified using the Spectramax microplate reader.

Nitrate/nitrite production

Nitrite production, an indicator of NO synthesis, was measured in the supernatant of J774 macrophages by using the Griess reaction.[18] Aliquots of supernatant (0.1 ml) were mixed with 0.1 ml of Griess reagent (1% sulfanilamide and 0.1% naphthyl ethylenediamine in 5% phosphoric acid). The optical density at 550 nm (OD_{550}) was measured using the Spectramax microplate reader. Nitrite concentrations were calculated by comparison with OD_{550} of standard solutions of sodium nitrite prepared in cultured medium.

In plasma samples, nitrate is the major degradation product of NO. Nitrate was converted to nitrite as described[18] by incubation with 60 mU nitrate reductase and 25 mM NADPH for 180 min. Nitrate was then measured as described above by the Griess reaction.

In vivo experiments

Male Wistar rats weighing 215–290 g were used for the experiments (from Charles River, Wilmington, MA, USA). Rats were maintained under controlled temperature and light cycle and fed *ad libitum*. All experiments were performed according to the National Institutes of Health guidelines for the use of experimental animals.

Animals were pretreated with a sublethal dose of LPS (1 mg/kg) or RD-LPS (1 mg/kg) intraperitoneally (i.p.) once a day for four consecutive days. At day 5, rats received a lethal dose of LPS (15 mg/kg i.p.). Rats were then sacrificed 6 h after the lethal injection of LPS and plasma samples, peritoneal macrophages and lungs were

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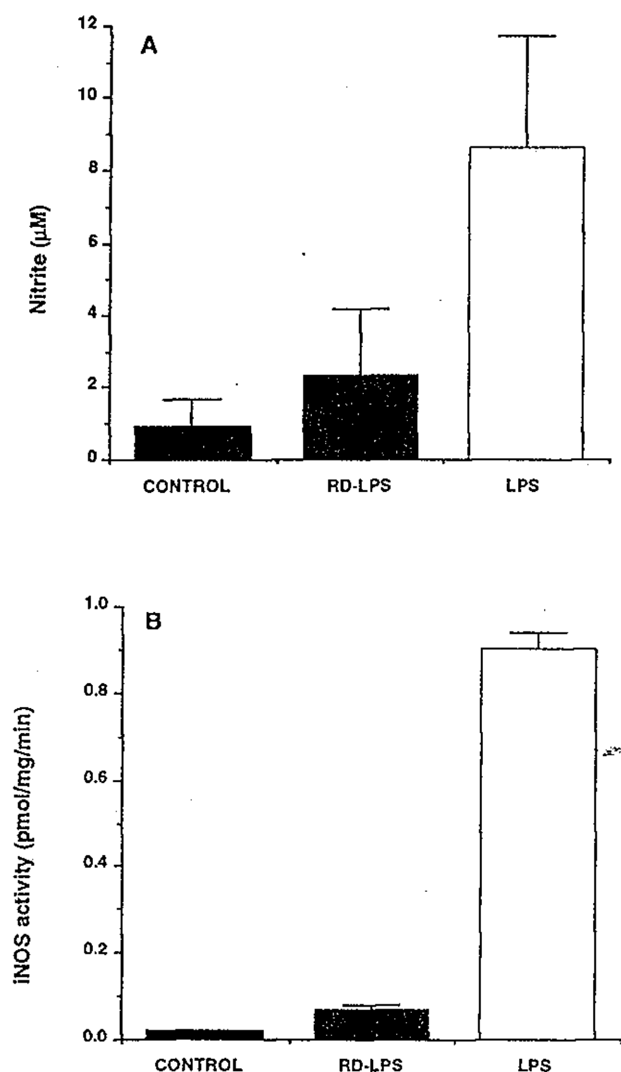

Fig. 1 Nitrite production (A) and iNOS activity (B) by J774 macrophages stimulated with DMEM only (control), RD-LPS or native LPS (10 ng/ml) for 24 h. Each value represents the mean ± SEM of n = 18 wells from three independent experiments. *$P <$ 0.01 versus control.

collected. Rats were sacrificed by exsanguination under sodium thiopentol (120 mg/kg, i.p) anesthesia.

Plasma samples were obtained for nitrite/nitrate measurement (performed as described above).

Peritoneal macrophages were harvested by peritoneal lavage with DMEM containing penicillin (100 units/ml), streptomycin (100 µg/ml) and heparin sodium (10 units/ml) and plated in 24-well plates at a concentration of 1 × 10^6 cells/ml and incubated for 2 h at 37°C under standard conditions. After incubation, the supernatant was collected for nitrite measurement.

Lungs were collected and stored at –70°C for iNOS activity measurement (see below).

NO synthase activity

The activity of inducible NOS was measured in J774 macrophages or in lungs harvested from rats by conversion of [^{3}H]-L-arginine to [^{3}H]-L-citrulline.[18] Briefly, after homogenization, samples were incubated in the presence of [^{3}H]-L-arginine (10 µM, 5 kBq/tube), NADPH (1 mM), calmodulin (30 nM), tetrahydrobiopterin (5 µM), and EGTA (2 mM) for 20 min at 22°C. Reactions were stopped by dilution with 0.5 ml of ice cold *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic (HEPES) buffer (pH 5.5) containing EGTA (2 mM) and EDTA (2 mM). Reaction mixtures were applied to Dowex 50W (Na^+ form) columns and the eluted [^{3}H]-L-citrulline activity was measured by a Wallac scintillation counter (Wallac, Gaithersburg, MD, USA).

Materials

Bacterial lipopolysaccharide of *E. coli* 089 and its radiodetoxified form were prepared at the Frédéric Joliot Curie National Research Institute for Radiobiology and Radiohygiene, Budapest, Hungary as previously described.[19] DMEM and fetal calf serum were obtained from Gibco (Grand Island, NY, USA). [^{3}H]-L-Arginine was obtained from DuPont/NEN (Boston, MA, USA). All other compounds and reagents were obtained from Sigma (St Louis, MO, USA).

Statistical analysis

All values in the text and in the figures are expressed as mean ± SEM on n observations, where n stands for number of animals or samples. Student's t test (unpaired) corrected by Fisher's test was used to compare means among and between groups. A P value of < 0.05 was considered to be statistically significant.

RESULTS

Effect of a subthreshold LPS or RD-LPS treatment on NO synthesis and NOS activity in J774 macrophages in vitro

In preliminary experiments, cells were treated for 24 h with various concentrations of LPS or RD-LPS (1 ng to 10 µg/ml) in order to determine an optimal subthreshold concentration to use for the induction of tolerance. At a concentration of 10 ng/ml, RD-LPS did not elicit a significant increase of nitrite production, while the native LPS induced a moderate but significant ($P < 0.01$) increment in comparison to control unstimulated cells (2.3 ± 1.9 µM, 8.6 ± 3.1 µM and 0.9 ± 0.8 µM, in RD-LPS, LPS and vehicle-treated cells, respectively; Fig. 1A). The increase of nitrite levels was associated with an increased expression of the iNOS enzyme in LPS-stimulated J774 cells. In

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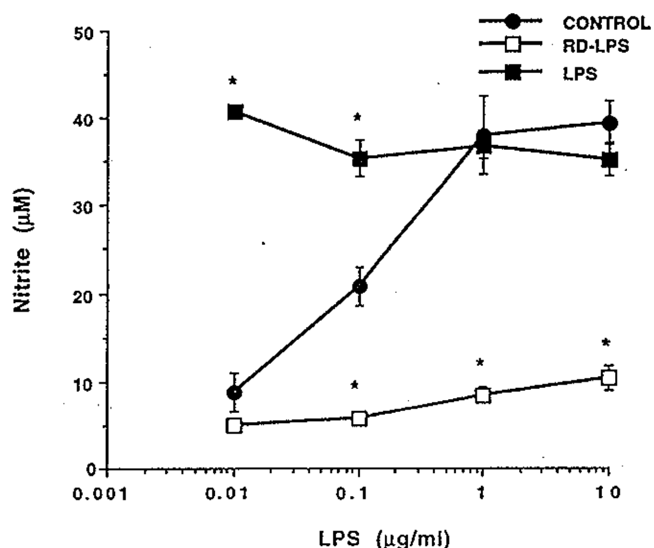

Fig. 2 Effect of RD-LPS or LPS pre-exposure on nitrite production by J774 macrophages. Cells were pretreated with DMEM only (control), RD-LPS or native LPS (10 ng/ml) for 24 h. After pretreatment, cells were stimulated with increasing concentrations of LPS (10 nM to 10 µg/ml). Each value represents the mean ± SEM of $n = 18$ wells from three independent experiments. *$P <$ 0.01 versus control.

contrast, iNOS activity of RD-LPS-stimulated cells was not significantly different from control (baseline) iNOS activity (Fig. 1B).

Effect of a subthreshold LPS or RD-LPS pretreatment on LPS-induced NO production in J774 macrophages in vitro

In subsequent studies, cells were pretreated with 10 ng/ml concentration of LPS or RD-LPS for 24 h. Cells were then washed with fresh medium and stimulated for a further period of 24 h with different concentrations of the same serotype of native LPS (1 ng to 10 µg/ml). As shown in Figure 2, pretreatment with a subthreshold concentration of RD-LPS caused a hyporesponsiveness to subsequent stimulation of LPS, since nitrite levels were similar to unstimulated cells at all LPS concentrations tested. In contrast, subthreshold concentration of LPS, applied as a pretreatment was not able to inhibit LPS-induced increase in nitrite levels, but, on the contrary, it even caused at enhancement of nitrite production, when lower concentrations of LPS ((0.01–0.1 mg/ml) were used in the second 24 h stimulation period (Fig. 2; $P < 0.01$ versus unstimulated and RD-LPS pretreated cells). Cell viability, as assessed by the measurement of mitochondrial respiration, was always ≥95% (data not shown).

Effect of a subthreshold LPS or RD-LPS pretreatment on LPS-induced TNF production in J774 macrophages in vitro

To investigate the specificity of the suppression of NO production by RD-LPS, we have also investigated the effect of RD-LPS pretreatment on the production of TNF. First, and identical to the experiments on NO production, cells were pretreated with a 10 ng/ml concentration of LPS or RD-LPS for 24 h. At this time point, TNF was measured in the supernatant. Cells were then washed with fresh medium and stimulated for a further period of 24 h with a second, higher dose of LPS (10 µg/ml). As shown in Figure 3, during the first 24 h, RD-LPS (10 ng/ml) caused a significantly lower production of TNF, when compared to LPS. However, after washouts, and over the second 24 h incubation period, both LPS and RD-LPS caused an enhancement of the production of TNF in response to LPS (Fig. 3). Thus, while pretreatment with RD-LPS rendered the cells hyporesponsive to NO production, the same pretreatment caused a hyperresponsiveness to TNF production.

NO synthesis in control and LPS or RD-LPS pretreated rats subjected to endotoxic shock

Since RD-LPS pretreatment in J774 cells altered the LPS-induced NO synthesis, in further experiments we sought

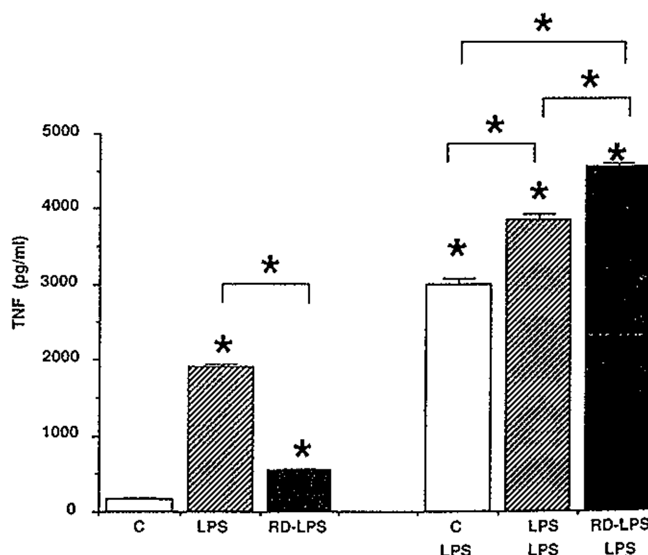

Fig. 3 Effect of RD-LPS or LPS pre-exposure on TNF production by J774 macrophages. Left bars: TNF production in cells pretreated with DMEM only (control), RD-LPS or native LPS (10 ng/ml) for 24 h. Right panels: TNF production in response to LPS (10 µg/ml) in cells pretreated with vehicle for 24 h, in cells pretreated with RD-LPS (10 ng/ml) for 24 h, followed by washouts, and in cells pretreated with LPS (10 ng/ml) for 24 h, followed by washouts. Each value represents the mean ± SEM of $n = 6$ wells from two independent experiments. *$P < 0.01$ versus control.

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to determine whether in vivo treatment with sublethal dose of LPS or RD-LPS modifies NO production during induction of endotoxic shock. For this purpose, rats were subjected to repeated administrations of LPS or RD-LPS (1 mg/kg/day i.p.) for four consecutive days. At the fifth day, rats were subjected to endotoxic shock by administration of a high dose of native *E. coli* LPS (15 mg/kg i.p., LD_{90}). 6 h after lethal LPS administration, animals were sacrificed and changes in NO synthesis were evaluated. Induction of endotoxic shock induced a significant elevation in plasma nitrate/nitrite levels and nitrite/nitrate production from peritoneal macrophages, in endotoxic shocked rats, when compared to control rats (Fig. 4A,B; $P < 0.01$). The increase in plasma and macrophage nitrate/nitrite levels was also associated with a significant induction of the inducible iNOS activity in the lungs of endotoxin shocked rats (Fig. 4C).

Pretreatment with repeated sublethal doses of RD-LPS inhibited increase of plasma nitrate/nitrite levels, NO production in peritoneal macrophages and induction of lung iNOS activity after lethal LPS challenge (Fig. 4). On the other hand, pretreatment with repeated sublethal doses of native LPS did not affect NO production during endotoxic shock (Fig. 4A–C).

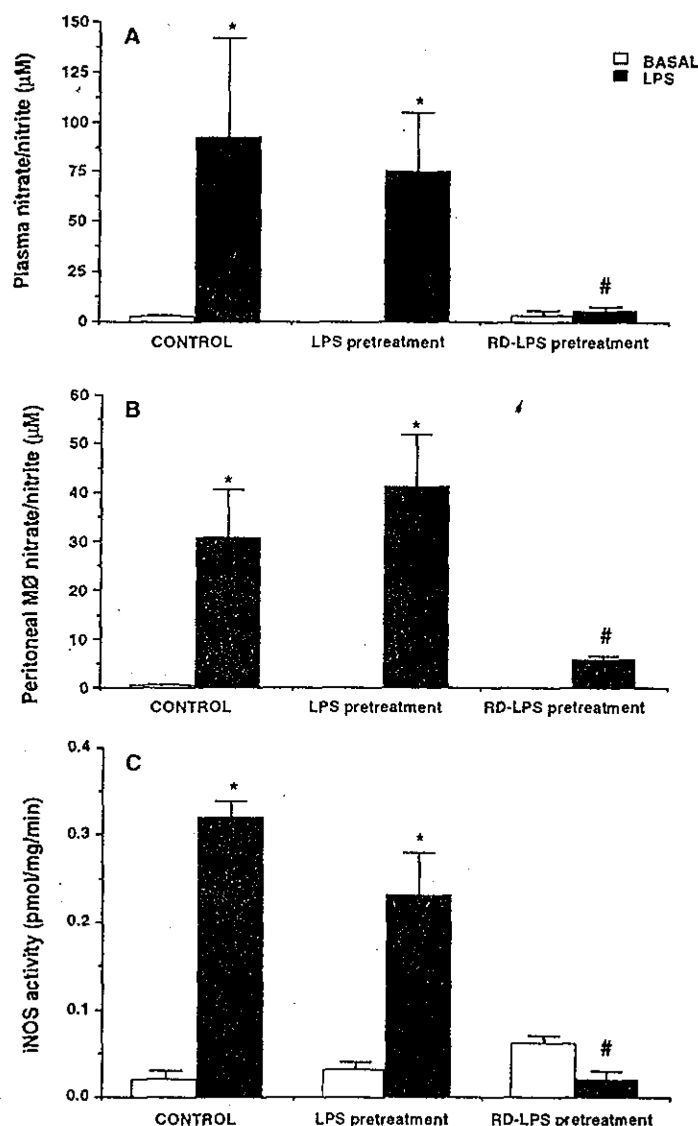

Fig. 4 Effect of in vivo pretreatment with RD-LPS or LPS on plasma nitrate/nitrite levels (A), peritoneal macrophage (Mø) nitrate/nitrite levels (B) and lung iNOS activity (C) in rats subjected to endotoxic shock. Rats, given sublethal doses of RD-LPS, LPS (1 mg/kg i.p.) or saline solution only (control) for four days, were challenged with a high dose of LPS (15 mg/kg i.p.) at the fifth day. Closed columns represent basal values before lethal LPS challenge; hatched columns represent values obtained 6 h after high-dose LPS challenge. Each value represents the mean ± SEM of five rats for each group. $*P < 0.01$ versus control. $\#P < 0.01$ versus LPS pretreatment.

DISCUSSION

It has been shown that ionizing (^{60}Co-γ) radiation significantly diminishes the undesirable toxic properties of native endotoxin while maintaining its well known immunoadjuvant, shock-preventing and nonspecific resistance enhancing capacities.[12] Pretreatment of experimental animals with RD-LPS has proven to be effective in preventing cardiovascular derangements and lethality in animal models of endotoxic and septic shock.[12–15] Furthermore, an interesting feature of RD-LPS pretreatment is its ability to induce tolerance to LPS-unrelated noxious stimuli. The latter include cross-tolerance to hemorrhagic shock and splanchnic ischemia and reperfusion injury.[12] The cellular mechanism of this non-specific host resistance is not yet completely defined. It is generally accepted, however, that the different biological activities of endotoxin (LPS) are initiated by a number of endogenous mediators, including NO, released from macrophages and other cell types.[3] The data described in this paper suggest that nonspecific resistance induced by RD-LPS may be acquired through modification of macrophage function. Our results show that pretreatment with RD-LPS reduces the ability of a toxic dose of LPS to induce iNOS activity and, consequently, NO production in the murine J774 macrophages. The down-regulation of NO synthesis was also observed in in vivo experiments in endotoxic shocked rats. Prior exposure to repeated sublethal doses of RD-LPS inhibited the plasma elevation of NO metabolites in rats challenged with a lethal dose of LPS. In vivo pretreatment with small doses of RD-LPS was also associated with a reduced capacity of LPS to induce iNOS in the lung and to trigger NO synthesis in peritoneal macrophages ex vivo.

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It is noteworthy, that in comparative in vitro and in vivo experiments, pretreatment with a small dose of native LPS was not able to blunt down the induction of iNOS in J774 macrophages and in endotoxic-treated rats. It appears that the RD-LPS and the native preparations tested in this study can function qualitatively in a different way. Several mechanisms could be at the basis of the phenomenon. The radiation alters the chemical structure of the endotoxin in the lipid fraction mainly, in the glucosamine contents and in the peptide fraction.[12,19] It is probable that these structural changes are the principal cause of the modification of RD-LPS-macrophage interactions at different levels such as at: (i) endotoxin interaction with LPS binding proteins and then with appropriate receptors; (ii) transmission of the intracellular signal through different biochemical pathways (i.e. tyrosine kinase activity or G protein involvement); and (iii) activation of transcription factors, such as nuclear factor kappa B. Another possibility is that RD-LPS may prevent LPS-induced NO synthesis by a simple competitive inhibition of native LPS activity with LPS-binding proteins or LPS receptors. The mechanism by which RD-LPS desensitizes LPS to induce iNOS in response to LPS requires further investigations.

To investigate the specificity of the suppression by RD-LPS of iNOS induction, we have also investigated whether or not RD-LPS renders the J774 macrophages tolerant to the LPS-induced production of TNF. Our results demonstrated that, in contrast to NO production, there was no tolerance against LPS-induced TNF production by RD-LPS pretreatment (Fig. 3). This is not entirely surprising, since the production of TNF and the induction of iNOS do not utilize the same intracellular pathways. Moreover, in cultured macrophages in vitro (as opposed to the in vivo situation in endotoxemia), the production of TNF does not precede or does not mediate the induction of iNOS.[2] Nevertheless, the finding that RD-LPS pre-exposure renders cells hyporesponsive to the production of one (NO) but not another (TNF) proinflammatory mediator, indicates that the current protocol does not cause a generalized state of hyporesponsiveness to the production of inflammatory mediators, and thus it may not be considered as 'classical' endotoxin tolerance.

In experimental models of in vitro or in vivo LPS tolerance, the role of NO is a subject of controversy. It has been demonstrated that NO synthesis can be up-regulated or down-regulated in murine macrophages simply modifying LPS concentration and the period of exposure.[21,22] Similar discrepancies have been observed after in vivo pretreatment with sublethal doses of LPS. Pre-exposure to repeated injections of LPS in rats attenuated the induction of iNOS by LPS in lungs;[23] whereas pretreatment with a single dose of LPS upregulated NO synthesis, the latter effect closely paralleled by an improvement in survival of tolerant rats subjected to endotoxic shock.[24,25] Therefore, it appears that repeated administration of LPS can exert either stimulatory or suppressive effects on NO synthesis depending on the experimental conditions (time and dose of exposure and, possibly, the type of LPS used). In the present model of endotoxemia, RD-LPS pretreatment caused a massive reduction in the induction of iNOS by LPS. There are now several reports that isoform-selective inhibition of iNOS offers a protection against the cardiovascular changes and lethality in rodent models of endotoxic shock.[2,26] Although it is tempting to speculate that suppression of iNOS induction by RD-LPS may contribute to the previously established protection conferred by RD-LPS administration against systemic inflammation and lethality in experimental endotoxin shock,[12] several other points should also be considered.

1. While in the current study, we have demonstrated that RD-LPS can render cells hyporesponsive to NO production, similar effects on TNF production were not observed. Thus, in vivo, the suppression of iNOS induction may have been associated with opposite changes in the production of other proinflammatory mediators. Clearly, the effect of RD-LPS on a variety of proinflammatory (and also antiinflammatory) mediators remains to be investigated.

2. While in the current study, we have observed that pretreatment of rats with RD-LPS reduces the induction of iNOS by LPS, similar reduction was not seen by pretreatment by native LPS. On the other hand, it is well known that native LPS pretreatment (similar to RD-LPS pretreatment) can also decrease mortality in response to subsequent, higher doses of LPS. Thus, there may not be a simple relationship between iNOS induction and mortality in endotoxin tolerance, inasmuch as endotoxin-induced mortality can be influenced by a variety of pro- and anti-inflammatory factors, iNOS being only one of them.

In conclusion, in the present report, we demonstrate that in vitro or in vivo pre-exposure to RD-LPS (but not to its native form) reduces the induction of NO synthesis in response to stimulation by a high-dose LPS in vitro and in vivo. It remains to be further investigated whether this effect may contribute to the previously observed beneficial effects of RD-LPS pretreatment in shock and, possibly, in other models of injury. Further delineation of the cellular mechanisms of this desensitization will provide important insights in understanding the development of the RD-LPS-mediated non-specific resistance.

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REFERENCES

1. Bone R.C. The pathogenesis of sepsis. *Ann Intern Med* 1991; 115: 457–469.
2. Szabó C. Alterations in nitric oxide production in various forms of circulatory shock. *New Horizons* 1995; 3: 2–32.
3. Nathan C. Nitric oxide as a secretory product of mammalian cells. *FASEB J* 1992; 6: 3051–3064.
4. Hoffman R.A., Langrehr J.M., Billiar T.R., Curran R.D., Simmons R.L. Alloantigen-induced activation of rat splenocytes is regulated by the oxidative metabolism of L-arginine. *J Immunol* 1990; 145: 2220–2226.
5. Wright C.E., Rees D.D., Moncada S. Protective and pathological roles of nitric oxide in endotoxin shock. *Cardiovasc Res* 1992; 28: 48–57.
6. Ziegler-Heitbrock H.W.L. Molecular mechanism in tolerance to lipopolysaccharide. *J Inflamm* 1995; 45: 13–26.
7. Greisman S.E. Induction of endotoxin tolerance. In: Nowotny A. (Ed) *Beneficial Effects of Endotoxins*. New York: Plenum, 1983; 149–178.
8. Ziegler-Heitbrock H.W.L., Blumenstein M., Käfferlein E. et al. In vitro desensitization to lipopolysaccharide suppresses tumor necrosis factor, interleukin-1 and interleukin 6 gene expression in a similar fashion. *Immunology* 1992; 75: 264–268.
9. Zingarelli B., Chen H., Caputi A.P., Cook J.A. Reorientation of macrophage mediator production in endotoxin tolerance. *Prog Clin Biol Res* 1995; 392: 529–537.
10. Moore J.N., Cook J.A., Morris D.D., Halushka P.V., Wise W.C. Endotoxin induced procoagulant activity, eicosanoid synthesis, and tumor necrosis factor production by rat peritoneal macrophages: Effect of endotoxin tolerance and glucan. *Circ Shock* 1990; 31: 281–295.
11. Randow F., Syrbe U., Meisel C. et al. Mechanism of endotoxin desensitization: involvement of interleukin 10 and transforming growth factor β. *J Exp Med* 1995; 181: 1887–1892.
12. Bertók L. Stimulation of nonspecific resistance by radio-detoxified endotoxin. In: Nowotny A. (Ed) *Beneficial Effects of Endotoxin*. New York: Plenum, 1983; 213–226.
13. Bertók L., Juhász-Nagy A., Sótonyi P. Prevention of cardiac damage induced by formyl-leurosine, a potent cytostatic agent by radio-detoxified endotoxin (Tolerin) in dogs. *Immunopharmacology* 1984; 8: 13–17.
14. Bertók L., Szeberényi S. Effect of radio-detoxified endotoxin on the liver microsomal drug metabolizing enzyme system in rats. *Immunopharmacology* 1983; 6: 1–5.
15. Bende Jr S., Bertók L., Bende Sen S. Protective effect of radio-detoxified endotoxin (Tolerin) on the ultrastructure of pancreas in experimental endotoxin shock of rats. *Acta Chir Hung* 1993; 33: 197–208.
16. Bánhegyi D., Várnai F., Horváth A., Bertók L. Treatment of severe immune deficiency with radio-detoxified endotoxin. *J Med* 1992; 23: 154–156.
17. Füst G., Bertók L., Juhász-Nagy S. Interactions of radio-detoxified *Escherichia coli* endotoxin preparations with the complement system. *Infect Immun* 1977; 16: 26–31.
18. Zingarelli B., O'Connor M., Wong H., Salzman A.L., Szabó C. Peroxynitrite-mediated DNA strand breakage activates poly-ADP ribosyl synthetase and causes cellular energy depletion in macrophages stimulated with bacterial lipopolysaccharide. *J Immunol* 1996; 156: 350–358.
19. Elekes E., Lüderitz O., Galanos Ch., Bertók L. Alterations in the chemical composition and antigenicity of *Escherichia coli* 089 lipopolysaccharide and lipid A after ^{60}Co gamma irradiation. *Acta Microbiol Hung* 1988; 35: 301–305.
20. Szabó C. The role of peroxynitrite in the pathophysiology of shock, inflammation and ischemia-reperfusion injury. *Shock* 1996; 6: 79–88.
21. Zhang X., Morrison D.C. Lipopolysaccharide-induced selective priming effects on tumor necrosis factor α and nitric oxide production in mouse peritoneal macrophages. *J Exp Med* 1993; 177: 511–516.
22. Severn A., Xu D., Doyle J. et al. Pre-exposure of murine macrophages to lipopolysaccharides inhibits the induction of nitric oxide synthase and reduces leishmanicidal activity. *Eur J Immunol* 1993; 23: 1711–1714.
23. Szabó C., Thiemermann C., Wu C.C., Perretti M., Vane J.R. Attenuation of the induction of nitric oxide synthase by endogenous glucocorticoids accounts for endotoxin tolerance in vivo. *Proc Natl Acad Sci USA* 1994; 91: 271–275.
24. Zingarelli B., Halushka V., Caputi P., Cook J.A. Increased nitric oxide synthesis during the development of endotoxin tolerance. *Shock* 1995; 3: 102–108.
25. Zingarelli B., Makhlouf M., Halushka P.V., Caputi A.P., Cook J.A. Altered macrophage function in tumor necrosis factor α- and endotoxin-induced tolerance. *J Endotoxin Res* 1995; 2: 247–254.
26. Southan G.J., Szabó C. Selective pharmacological inhibition of distinct nitric oxide synthase isoforms. *Biochem Pharmacol* 1996; 51: 383–394.

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