In Vivo Inhibition of Lipopolysaccharide-Induced Lethality and Tumor Necrosis Factor Synthesis by *Rhodobacter sphaeroides* Diphosphoryl Lipid A Is Dependent on Corticosterone Induction

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Diphosphoryl lipid A from the lipopolysaccharide (LPS) of *Rhodobacter sphaeroides* (Rs-DPLA) has been demonstrated to block in mice and guinea pigs the increase in the serum tumor necrosis factor (TNF) response induced by highly purified deep rough chemotype LPS from *Escherichia coli* D31m4 (ReLPS). The present study was designed to determine the role of corticosterone induction by Rs-DPLA and its effect on TNF regulation and survival in lethal endotoxin shock models and to evaluate the ability of Rs-DPLA to induce endotoxin tolerance. Administration of a 100-fold excess of Rs-DPLA 1 h prior to ReLPS administration inhibited the characteristic peak in serum TNF levels induced by LPS. Inhibition was apparent in normal and D-galactosamine (GaIN)-sensitized mice and occurred at the pretranslational level, as splenic TNF and interleukin-1β mRNAs were present in lower amounts in ReLPS-stimulated mice pretreated with Rs-DPLA. Consistent with its effects in reducing serum TNF levels, Rs-DPLA pretreatment protected GaIN-sensitized mice from a lethal ReLPS challenge. In contrast, Rs-DPLA did not inhibit the increase in the serum TNF response or protect against a lethal ReLPS challenge in parallel experiments with adrenalectomized (Adrex) mice, for which the 50% lethal dose of ReLPS was comparable to that for GaIN-sensitized mice. Furthermore, Rs-DPLA appeared to prime Adrex animals and increase the magnitude of the serum TNF response to a suboptimal LPS stimulus. Priming by Rs-DPLA, however, was not observed in normal or GaIN-sensitized mice. Although Rs-DPLA by itself was nontoxic and unable to elevate serum TNF levels in any of the models investigated, it did induce a significant increase in the serum corticosterone response and was capable of inducing endotoxin tolerance in normal mice. The inability of Rs-DPLA to protect Adrex mice from a lethal ReLPS stimulus or to inhibit the increase in the serum TNF response suggests that the protective effect of Rs-DPLA in normal or GaIN-sensitized animals occurs through corticosterone induction. These results support the concept that endogenous glucocorticoids can modulate the endotoxic effects of LPS by inhibiting the synthesis of inflammatory cytokines.

Endotoxic lipopolysaccharides (LPS) from the cell walls of gram-negative bacteria play a fundamental role in the pathology associated with sepsis and septic shock. Many of the sequelae associated with endotoxin shock have been demonstrated to occur via the induction of inflammatory cytokines, including interleukin-1 (IL-1) and tumor necrosis factor (TNF) (7). The demonstration that an IL-1 receptor antagonist protein or antibodies against TNF protect animals against the lethal effects of endotoxin suggests the importance of these cytokines in the acute inflammatory responses associated with endotoxemia (4, 9, 26, 42).

Endotoxin shock has been investigated with numerous animal models, and species differences related to endotoxin sensitivity as well as to the protective effects of exogenous glucocorticoids have been reported (9, 43–45, 48). Although mice are relatively resistant to the lethal effects of endotoxin in comparison with rabbits or sheep, for example, rodent models with an increase of several orders of magnitude in their sensitivity to bacterial endotoxin have been developed (3, 11, 14, 16, 48). Two such models are adrenalectomized (Adrex) mice and D-galactosamine (GaIN)-sensitized mice. In the former model, Adrex mice do not have any detectable serum corticosterone, whereas in the latter model, hepatic function is significantly compromised (5). These models enable a more comprehensive understanding of the inflammatory events associated with endotoxin shock and the resulting compensatory responses elicited by the host. Furthermore, rodent endotoxin shock models may contribute to the identification of endotoxin antagonists useful for the treatment of septic shock.

The development of nontoxic lipid A analogs has been the goal of numerous laboratories in an attempt to dissociate the inflammatory aspects of bacterial endotoxin from its adjuvant or immunostimulatory properties. A monophosphoryl lipid A derivative, for example, has been reported to be significantly less toxic or pyrogenic than the native endotoxin from *Salmonella typhimurium* (34). This molecule, while capable of inducing endotoxin tolerance, also stimulates increases in serum colony-stimulating factor, interferon, IL-6, and TNF responses in mice (19, 24). A nontoxic diphosphoryl lipid A species, pentaacyl Rs-DPLA, that did not stimulate TNF production by RAW264 cells or induce TNF in vivo following injection into BALB/C mice was isolated from *Rhodobacter sphaeroides* (33, 40). Rs-DPLA...
could, however, compete with Escherichia coli deep rough chemotype LPS (Rs-DPLA) and significantly mitigate the induction of TNF in vitro or in vivo (31, 33, 40). Although the mechanism by which Rs-DPLA inhibited the increase in the TNF response in vitro was not known, the possibility that Rs-DPLA was functioning as an endotoxin antagonist seemed likely. However, whether the protective effect of Rs-DPLA in vivo occurred through a similar pathway, with Rs-DPLA functioning as a receptor antagonist, was unclear and required further investigation.

In the present study, we used the LPS antagonist Rs-DPLA (Fig. 1B) and a well-characterized toxic LPS, ReLPS (Fig. 1A), to investigate the mechanism of endotoxicity of LPS in mice as well as the compensatory response of the host. While Rs-DPLA suppressed the increase in the serum TNF response in normal and GalN-sensitized mice, it failed to block the increase in the serum TNF response in ReLPS-stimulated Adrex mice. These results were consistent with the inability of Rs-DPLA to protect Adrex animals from a lethal dose of ReLPS while protecting GalN-sensitized mice from a comparable challenge. Rs-DPLA induced corticosterone in normal mice. This result, coupled with those of the Adrex mouse studies, suggests that the in vivo protective effect of Rs-DPLA occurs primarily through corticosterone induction.

MATERIALS AND METHODS

Preparation of ReLPS and Rs-DPLA. R. sphaeroides ATCC 17023 was grown photoheterotrophically for 12 to 14 days and harvested by centrifugation. LPS was extracted with phenol-chloroform-petroleum ether and prepared as previously described (30, 33). Rs-DPLA was prepared by treating R. sphaeroides LPS with 0.02 M sodium acetate (pH 2.5) for 70 min at 100°C. Crude Rs-DPLA was dissolved in chloroform-methanol and purified by DEAE-cellulose column chromatography. Pentaacetyl Rs-DPLA was eluted with a linear ammonium acetate gradient (33) and characterized by analytical thin-layer chromatography, gas-liquid chromatography, mass spectrometry, and nuclear magnetic resonance spectroscopy as previously described (30, 33). ReLPS (hexaacyl) from E. coli D31m4 was purified as described previously (32). Both Rs-DPLA (Fig. 1B) (33) and ReLPS (Fig. 1A) (32, 41) were in the free acid form, were solubilized in 0.5% triethylamine at 10 mg/ml, generating the triethylamine salt, and were further diluted with saline prior to injection. In experiments involving Rs-DPLA priming in GalN-sensitized and Adrex mice, ReLPS was directly suspended in saline, as was E. coli O55:B5 LPS (Difco, Detroit, Mich.), which was used as an additional control.

Endotoxin models. Normal and Adrex female BALB/c mice were obtained from Charles River Laboratories. Animals were housed for at least 1 week after surgery before use. Mice were sensitized to GalN by intravenous (i.v.) injection of 8 to 10 mg of GalN coincident with ReLPS (11, 14, 16). In both the Adrex and the GalN-sensitized mouse models, the 50% lethal dose of ReLPS was shifted to greater than 400 µg per animal to 10 to 20 µg. In normal, GalN-sensitized, and Adrex mouse models, Rs-DPLA at concentrations of 0.1 to 2 mg per animal was injected intraperitoneally (i.p.) 1 h prior to an i.v. or i.p. injection of 10 to 20 µg of ReLPS. Animals were bled at various intervals for TNF and corticosterone measurements. Alternatively, animals were monitored for 72 h to determine the effects of Rs-DPLA pretreatment on ReLPS-mediated lethality. Oral dexamethasone (1 mg/kg) pretreatment was used as a positive control for inhibiting the increase in the serum TNF response and the increased mortality following ReLPS challenge. All animal experiments were performed in accordance with National Institutes of Health guidelines for the use of experimental animals.

TNF and corticosterone assays. Mice were bled 1 h after ReLPS injection, and serum dilutions of between 0.5 and 2% were assayed by the murine L929 fibroblast cytotoxicity assay as previously described (9). L929 cells were plated in microtitre plates at 5 × 10^4 cells per well containing 100 µl of RPMI 1640 medium with 10% fetal calf serum (GIBCO, Grand Island, N.Y.) and 1 µg of actinomycin D per ml. Following overnight incubation, plates were stained with 0.5% crystal violet in 25% methanol and rinsed, and the A_570
was read on a Dynatech MR600 microtiter plate reader. Recombinant murine TNF (provided by Lee Bobbit, Lilly Research Laboratories) was used for a standard curve. The conversion of optical densities to picograms of TNF per milliliter was based on the serum dilutions that fell on the linear portion of the standard curve. Serum corticosterone levels were measured in serum 3 h after i.p. injection of Rs-DPLA or ReLPS by radioimmunoassay (Radioassay Systems Laboratories, Carson, Calif.). Unless otherwise indicated, all reagents were from Sigma (St. Louis, Mo.).

RNA analysis. Spleens from three or four mice per group were pooled 1 h after ReLPS injection, RNA was prepared following guanidine thiocyanate lysis and cesium chloride gradient centrifugation, and 5 μg per slot was blotted on nitrocellulose filters (11). mRNA was quantitated with 5'-end-labeled oligonucleotide probes specific for murine TNF-α and IL-1β (Amgen, Thousand Oaks, Calif.) and was normalized by hybridization with an oligo(dT) probe (Promega, Madison, Wis.). Hybridization was quantitated with a Betascope 603 blot analyzer (Betagen Corp., Waltham, Mass.), which provided counts for each slot prior to autoradiography.

RESULTS

Effect of Rs-DPLA on the induction of TNF and IL-1β in mice. The central role of TNF in mediating the toxic effects of LPS (4) necessitated an evaluation of the effect of Rs-DPLA pretreatment and ReLPS challenge on the induction of TNF in normal, GalN-sensitized, and Adrex mice as three distinct models of endotoxin shock. In agreement with a previous study with normal mice (31), prophylactic administration of Rs-DPLA blocked the increase in the serum TNF response (>95%) when measured 1 h after ReLPS injection in normal and GalN-sensitized mice (Fig. 2). The inhibition observed in both models was most effective when mice were pretreated with a 100-fold excess (by weight) of Rs-DPLA over the ReLPS challenge. Significant inhibition of the ReLPS-induced increase in the serum TNF response was also observed in animals pretreated orally with 1 mg of dexamethasone per kg (Fig. 2).

While Rs-DPLA pretreatment inhibited the increase in the serum TNF response, it was necessary to determine whether the inhibition was at the translational or pretranslational level. Splenic RNA from mice treated with Rs-DPLA, ReLPS, ReLPS plus Rs-DPLA, and ReLPS plus dexamethasone was probed for changes in TNF and IL-1β mRNA levels (Fig. 3). Increases in IL-1β and TNF mRNA levels in ReLPS-stimulated mice were apparent, while mice pretreated with Rs-DPLA prior to ReLPS had a 90% reduction in the amount of serum TNF and 70 and 34% reductions in the amounts of TNF and IL-1β mRNAs, respectively. Rs-DPLA treatment by itself did not result in a significant increase in splenic TNF or IL-1β mRNA levels.

Effect of Rs-DPLA pretreatment on ReLPS lethality in GalN-sensitized and Adrex mice. The inhibition of the ReLPS-mediated increase in the serum TNF response in GalN-sensitized mice suggested that Rs-DPLA could protect these animals from a lethal LPS challenge. As demonstrated in Fig. 4A, Rs-DPLA protected GalN-sensitized mice against an LD₅₀ of ReLPS, with survival in the Rs-DPLA-treated group being similar to that in the dexamethasone-treated group. Similar experiments were performed with Adrex mice to determine whether the protective effect of Rs-DPLA was apparent in other endotoxin models characterized by an increased sensitivity to LPS. However, in contrast to the protective effect of Rs-DPLA in GalN-sensitized mice, Rs-DPLA did not significantly increase the survival of Adrex mice subjected to a comparable ReLPS challenge (Fig. 4B). Dexamethasone (1 mg/kg) pretreatment was effective, with 100% survival being observed in both the Adrex and the GalN-sensitized mouse models.

Effect of Rs-DPLA on TNF levels in Adrex mice. Consistent with the inability of Rs-DPLA to protect Adrex mice from a lethal ReLPS challenge, Rs-DPLA pretreatment did not block the ReLPS-induced increase in the serum TNF response in Adrex mice (Table 1). Rs-DPLA pretreatment at a 100-fold excess over ReLPS doses of between 5 and 20 μg did not result in any reduction in the serum TNF response,
these agents in combination resulted in a significant increase in the TNF response (Table 2). A similar result was apparent when Rs-DPLA-treated Adrex mice were challenged with a suboptimal concentration of a more potent endotoxin preparation derived from *E. coli* O55:B5. Therefore, the synergy or priming effect of Rs-DPLA in Adrex mice observed when suboptimal ReLPS concentrations were used was not unique to the ReLPS but was observed for other LPS species as well. The priming effect observed with Rs-DPLA, however, was specific for Adrex mice and was not apparent in GalN-sensitized animals (Table 2).

**Induction of corticosterone by Rs-DPLA in normal mice.** The inability of Rs-DPLA to increase survival or inhibit an increase in the serum TNF response in Adrex mice suggested the possibility that the protective effect of Rs-DPLA in normal or GalN-sensitized mice might result from corticosterone induction. As demonstrated in Fig. 5, a significant increase in the serum corticosterone response was apparent 3 h after i.p. injection of Rs-DPLA, although ReLPS consistently resulted in higher corticosterone levels. The increase in the serum corticosterone response was not an artifact of

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**TABLE 2. Priming by Rs-DPLA of Adrex mice to respond to a suboptimal LPS challenge**

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>TNF, in pg/ml (SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ReLPS (10 μg)</td>
<td>&lt;100</td>
</tr>
<tr>
<td>Rs-DPLA (1 mg)</td>
<td>&lt;100</td>
</tr>
<tr>
<td>ReLPS + Rs-DPLA</td>
<td>4,594 (1,246)</td>
</tr>
<tr>
<td>LPS (0.5 μg)</td>
<td>&lt;100</td>
</tr>
<tr>
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<td>3,346 (1,069)</td>
</tr>
<tr>
<td>ReLPS (10 μg) (GalN)</td>
<td>&lt;100</td>
</tr>
<tr>
<td>ReLPS (GalN) + Rs-DPLA</td>
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</tbody>
</table>

*Adrex mice were injected i.p. with Rs-DPLA 1 h prior to an i.p. ReLPS challenge. Animals were bled 1 h after ReLPS or LPS administration or, in mice injected with Rs-DPLA alone, 1 h after Rs-DPLA administration. Note that both ReLPS and *E. coli* O55:B5 LPS were suspended in saline rather than triethylamine.

b *E. coli* O55:B5 LPS.

Mice were sensitized by injection of 10 mg of GalN coincident with the ReLPS injection.

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**TABLE 1. Effect of Rs-DPLA on ReLPS-induced TNF levels in Adrex mice**

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>TNF, in pg/ml (SEM), induced by the following ReLPS concn, in μg:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>ReLPS</td>
<td>3,516 (807)</td>
</tr>
<tr>
<td>ReLPS + Rs-DPLA</td>
<td>8,878 (1,087)</td>
</tr>
<tr>
<td>ReLPS + dexamethasone&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
</tr>
</tbody>
</table>

<sup>a</sup> ReLPS was injected i.v. 1 h after i.p. injection of a 100-fold excess of Rs-DPLA. ND, not determined.

<sup>b</sup> Dexamethasone at 1 mg/kg was given orally 1 h prior to the 20-μg i.v. injection of ReLPS.

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**FIG. 4.** Effect of Rs-DPLA on a lethal ReLPS challenge in GalN-sensitized and Adrex mice. (A) Mice (six per group) were injected with 1 mg of Rs-DPLA (DPLA) or vehicle or dosed orally with 1 mg of dexamethasone (Dex) per kg 1 h prior to an i.p. injection of 10 μg of triethylamine-solubilized ReLPS and 10 mg of GalN. (B) A similar protocol was used for Adrex mice, although the ReLPS challenge was performed in the absence of GalN. The survival of both GalN-sensitized and Adrex mice was monitored over the ensuing 48 h, with no further deaths being observed after 24 h in either group. The results from two separate experiments were pooled (12 mice total per experimental group) and expressed as percent survival relative to that at the time-zero point.

**FIG. 5.** Induction of the serum corticosterone response by Rs-DPLA. Mice (three per group) which had been conditioned for 1 to 2 weeks with constant handling were injected i.p. with vehicle, 1 mg of Rs-DPLA (DPLA), or 20 μg of ReLPS, sacrificed 3 h later, and bled by cardiac puncture, and the serum corticosterone response was quantitated by a radioimmunoassay. Brackets indicate the standard deviation. Shown is a representative experiment of three.

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whereas dexamethasone was effective. The apparent increase in the serum TNF response with the 5-μg ReLPS challenge suggested the possibility that Rs-DPLA may actually act in synergy with suboptimal doses of ReLPS and augment serum TNF levels in Adrex mice. This possibility was evaluated by injecting ReLPS suspended in saline by the i.p. route, which requires concentrations higher than those of triethylamine-solubilized ReLPS to obtain a TNF response comparable to that achieved by the i.v. route (44a). Whereas Rs-DPLA or ReLPS alone failed to induce TNF,
the injection procedure, as demonstrated by the injection of comparable volumes of vehicle to littermates.

Induction of endotoxin tolerance by Rs-DPLA. The ability of Rs-DPLA to induce corticosterone without an increase in the serum TNF response, coupled with the recent finding that endotoxin tolerance is dependent in part on endogenous glucocorticoids (11), suggested the possibility that Rs-DPLA was capable of inducing an endotoxin-tolerant state. As seen in Fig. 6, mice pretreated with Rs-DPLA 20 h prior to an ReLPS stimulus were endotoxin tolerant, and these animals did not show any significant increase in the serum TNF response 1 h after the ReLPS challenge. This result was comparable to those obtained with animals made endotoxin tolerant by pretreatment with ReLPS 20 h prior to a second ReLPS stimulus. In both instances, TNF levels were approximately 10-fold lower than those in mice receiving only the initial ReLPS stimulus. Therefore, while being unable to directly induce TNF, Rs-DPLA pretreatment resulted in an endotoxin-tolerant state in normal mice, presumably by a corticosterone-dependent process.

DISCUSSION

The development of structurally related analogs of toxic LPS and lipid A that lack inflammatory properties and yet function as possible endotoxin antagonists may provide useful therapeutic modalities for the treatment of septic shock. Strittmatter et al. first demonstrated that LPS from R. sphaeroides ATCC 17023 is 4 orders of magnitude less toxic in GalN-sensitized mice than LPS from Salmonella abortus-equii (39). Rs-DPLA isolated from this LPS did not induce TNF in vivo or in vitro and blocked TNF induction by ReLPS in mice and at the cellular level in human monocytes, murine macrophages, and cell lines (17, 31, 33, 40). Furthermore, this analog of toxic lipid A was inactive in inducing superoxide release from phorbol myristate acetate-primed alveolar macrophages (33) and had only slight stimulatory effects on the pre-B cell line 70Z/3 (20, 33). Rs-DPLA was also an effective antagonist in vitro in monocyte-macrophage cell populations and blocked both ReLPS induction of 70Z/3 cells (20) and stimulation of CD11b/CD18 expression on human neutrophils (23).

Previous studies reported on endotoxin analogs that were either agonists or antagonists. Monophosphoryl lipid A was a less potent inducer of TNF, IL-6, and interferon than either LPS or toxic lipid A and yet induced endotoxin tolerance (19, 24). The disaccharide lipid A precursor IVA was demonstrated to antagonize LPS induction of IL-1 in human monocytes (22) but to have agonist activity in 70Z/3 cells (38). Decacylated LPS was reported to function as an antagonist in the inhibition of LPS-stimulated neutrophil adhesion to human endothelial cells (28, 35) and LPS-induced mitogenesis of murine splenocytes (8). The ability of these endotoxin analogs to function as antagonists in vivo and protect animals from lethal endotoxemia remains to be determined. The monosaccharide lipid A precursor lipid X was reported to protect mice against lethal endotoxemia (29), suggesting that protection can be achieved through endotoxin antagonism, although immunostimulating contaminants in synthetic lipid X preparations were present (2). In later studies, chemically pure lipid X devoid of the contaminating disaccharide was used and found to protect GalN-sensitized mice from lethal endotoxemia (21).

In the present study, the mechanism by which Rs-DPLA protects mice from an LPS challenge was investigated with normal, GalN-sensitized, and Adrex animals. The protective effect of Rs-DPLA on normal mice induced by Rs-DPLA was not limited to a reduction in only TNF levels. Splenic mRNA studies demonstrated reduced IL-1β mRNA levels, suggesting pretranslational regulation of this inflammatory cytokine as well. These data are consistent with the previous in vitro observation that Rs-DPLA significantly inhibited the increase in ReLPS-induced thymocyte costimulatory activity (31). Rs-DPLA could function in vivo by antagonizing endotoxin receptors, inducing a compensatory antiinflammatory response, and/or inducing a state of endotoxin tolerance, the last possibility being demonstrated for monophosphoryl lipid A (19, 24) and low-dose endotoxin in GalN-sensitized mice (14). While the present results cannot exclude a role for Rs-DPLA in antagonizing macrophage endotoxin receptors, the inability to inhibit the ReLPS-induced increase in the serum TNF response in Adrex mice suggests that the protective effect of Rs-DPLA is mediated largely through corticosterone induction. However, induction of corticosterone is not the only mechanism by which Rs-DPLA inhibits increases in serum TNF responses. The protective effect of Rs-DPLA was mitigated when the ratio of Rs-DPLA to ReLPS was decreased to 50:1 or 25:1 or when less purified commercial LPS preparations were used in vivo (44a). Therefore, while the in vivo protective effects of Rs-DPLA can be related to its potential activity as an endotoxin receptor antagonist, the in vivo protective effects presumably involve receptor antagonism, corticosterone elevation, and tolerance induction. Inhibiting any one of these effects of Rs-DPLA, as with adrenalectomy, compromises the in vivo protective effects of Rs-DPLA. However, in contrast to its protective effects, Rs-DPLA at 1 mg per animal, although unable to induce a serum TNF response, did prime Adrex animals to respond to a suboptimal LPS challenge with a significant elevation in the serum TNF response. A similar priming was observed in a recent study when Adrex mice were pretreated with muramyl dipeptide 3 to 6 h prior to LPS challenge (27). The mechanism by which Rs-DPLA primes Adrex mice for an endotoxin challenge and whether priming by muramyl dipeptide occurs through a similar pathway are unknown at present.

LPS-mediated increases in serum IL-1 and TNF responses can be reduced by pretreatment of animals with pharmacologic agents, including glucocorticoids (9, 43, 45, 48), gold salts (10), pentoxifylline (36), and chlorpromazine (15), by
selective depletion of macrophages with dichloromethylene diphosphonate-containing liposomes (6), by injection of IL-6 (1) or monoclonal antibodies against TNF (13, 37, 42, 46), and by induction of endotoxin tolerance (11, 12, 18, 25, 47). While these agents and procedures may work through multiple levels, a central theme involving the disruption of macrophage-endotoxin interactions and the activation of the hypothalamic-pituitary-adrenal axis remains fundamental in the regulation of the cytokine response to inflammatory mediators. Previous studies showed the importance of endogenous glucocorticoids in mitigating an inflammatory response, as demonstrated by the increased sensitivity of Adrex animals compared with normal animals to endotoxin, IL-1, TNF, and bacterial sepsis (3, 9, 11, 48). The ability of exogenously administered glucocorticoids (9, 11, 15, 27, 43, 45, 48) to inhibit the LPS-mediated increase in the serum TNF response and mortality in various animal models further indicates their role in modulating the acute inflammatory response associated with endotoxemia.

Freudenberg and Galanos (14) demonstrated that pretreatment of mice with a sublethal LPS stimulus of 10 to 100 ng 1 to 50 h prior to challenge with 1,000 times the 50% lethal dose of LPS plus GalN resulted in endotoxin tolerance, with 100% survival being observed in the pretreated animals. The protective effect of Rs-DPLA demonstrated in GalN-sensitized mice (Fig. 4) and the absence of a TNF response in Rs-DPLA-pretreated animals are consistent with their observations. Furthermore, their demonstration that tolerance was dependent on LPS-responsive macrophages injected into C3H/HeJ mice provides further support for a macrophage-neuroendocrine axis resulting in corticosterone induction and contributing to the establishment of endotoxin tolerance.

In addition to direct transcriptional and translational effects on cytokine synthesis, glucocorticoids may downregulate cytokine synthesis by the induction of other mediators, possibly liver derived, which result in the establishment of an endotoxin-tolerant state. In normal animals, Rs-DPLA induces corticosterone with no detectable increase in the serum TNF response or splenic TNF mRNA levels. Therefore, endotoxin tolerance can be achieved without the necessity of inducing the initial increase in the serum TNF response. A similar conclusion was reached in studies with Adrex mice and glucocorticoids (11) and in a sepsis model involving cecal ligation and puncture (9). The present study further demonstrates that the hypothalamic-pituitary-adrenal axis is involved in the host response to lethal or sublethal concentrations of LPS. Interruption of this axis can have a profound effect on the response of the animal to a sublethal LPS challenge from a beneficial tolerance-inducing effect (with normal animals) to a potential detrimental effect (with Adrex animals) if endotoxin priming occurs. While the ability of Rs-DPLA to trigger the hypothalamic-pituitary-adrenal axis is clear, the mechanism by which Rs-DPLA induces corticosterone and not TNF or other inflammatory cytokines requires further investigation.

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REFERENCES

and monophosphoryl lipid A that result in equivalent early endotoxin tolerance. Infect. Immun. 58:2429–2437.


