INFECTION AND IMMUNITY, July 2005, p. 4238–4244 Vol. 73, No. 7
0019-9567/05/$08.00+0 doi:10.1128/IAI.73.7.4238–4244.2005
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Endocrine Perturbation Increases Susceptibility of Mice to Anthrax Lethal Toxin

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Received 30 December 2004/Returned for modification 12 February 2005/Accepted 26 February 2005

***Bacillus anthracis* lethal toxin (LT) causes vascular collapse and high lethality in BALB/cJ mice, intermediate lethality in C57BL/6J mice, and no lethality in DBA/2J mice. We found that adrenalectomized (ADX) mice of all three strains had increased susceptibility to LT. The increased susceptibility of ADX-DBA/2J mice was not accompanied by changes in their macrophage sensitivity or cytokine response to LT. DBA/2J mice showed no change in serum corticosteroid levels in response to LT injection, while BALB/cJ mice showed a fivefold increase in serum corticosterone. However, LT inhibited dexamethasone (DEX)-induced glucocorticoid receptor gene activation to similar extents in all three strains. DEX treatment did not rescue ADX mice from LT-mediated mortality. Surprisingly, oral DEX treatment also sensitized adrenally intact DBA/2J mice to LT lethality at all doses tested and also exacerbated LT-mediated pathogenesis and mortality in BALB/cJ mice. Aldosterone did not protect ADX mice from toxin challenge. These results indicate that susceptibility to anthrax LT in mice depends on a fine but easily perturbed balance of endocrine functions. Thus, the potentially detrimental consequences of steroid therapy for anthrax must be considered in treatment protocols for this disease.**

Anthrax lethal toxin (LT) consists of two polypeptides. The protective antigen (PA) binds to cellular receptors and translocates lethal factor (LF) into the cytosol (12). LT injection alone can induce the vascular collapse seen in anthrax (6, 14, 18, 25, 31). Macrophages from a subset of inbred mice are uniquely sensitive to LT-induced rapid lysis (19, 53), resulting in a characteristic cytokine response to the toxin (31, 32) that can contribute to higher sensitivity of some mouse strains (32). However, the correlation between macrophage sensitivity and animal susceptibility to LT described in early studies (22, 57) does not hold for other LT-susceptible species (which have LT-resistant macrophages) or for mice such as the relatively LT-resistant C3H/HeJ mice (with LT-sensitive macrophages) (32). Recent work has shown that additional genetic loci and numerous factors control LT susceptibility (29, 32).

LF is a metalloproteinase that cleaves and inactivates members of the mitogen-activated protein kinase family (MEKs) (16, 35, 51), but the consequences of MEK cleavage have not yet been linked with LT toxicity in cells or animals. LT also inhibits glucocorticoid receptor (GR) transactivation in a noncompetitive ligand-independent fashion (56).

GR binds glucocorticoids (GCs), the functional effector endpoint of the hypothalamic-pituitary-adrenal (HPA) axis and important modulators of the immune system. Infection, toxic insults, inflammatory cytokines, and other stress stimuli activate the HPA axis and induce adrenal GC synthesis and release (21–22). GCs, in turn, provide negative feedback to shut down the HPA axis (15, 42, 45, 48, 54, 55). At the molecular level, GCs bind to cytosolic GRs, which function as transcription factors after translocation to the nucleus (4, 7, 55). GRs also function as transcriptional repressors by interfering with other transcription factors, such as NF-κB and activator protein 1 (AP-1) (1, 24, 30), and exert their anti-inflammatory actions. GCs and GR also play an important role in maintaining cardiovascular homeostasis and glucose metabolism. GR is critical for life, as evidenced by lethality of GR knockout mice (11).

Strain differences in GC responsiveness are associated with susceptibility and resistance to inflammation in many species and inbred rodent strains (40, 41, 45). Hyper-HPA axis and GC responsiveness have been associated with resistance to inflammatory disease, while a blunted HPA axis and GC response are associated with greater susceptibility to some autoimmune/inflammatory diseases (8, 40, 41, 59). BALB/c mice and Fischer rats are hyper-GC responsive and resistant to inflammatory disease compared to hypo-HPA-responsive C57BL/6 mice or Lewis rats (2, 3, 8, 37, 38, 40, 41, 59). Interruption of the GC response by adrenalectomy (ADX) or with a GR antagonist (RU486) enhances the inflammatory responses through inhibition of GR-mediated cytokine gene repression and increases mortality in otherwise inflammation-resistant hosts. Increased susceptibility can usually be reversed by reintroduction of natural or synthetic corticosteroids (for a review, see reference 54).

Unlike the inverse relationship observed between HPA axis responsiveness and susceptibility to bacterial shock, hyper-HPA-responsive BALB/cJ mice and Fischer rats are sensitive to LT lethality (17, 31), while hypo-HPA-responsive C57BL/6J and DBA/2J mice and Lewis rats are relatively resistant (32). These correlations, along with the discovery that LT targets the

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GR activation pathway in cells and mice (56), led us to further investigate the effects of GCs and the HPA axis manipulations on LT-mediated toxicity. The data we report here show that neuroendocrine balance and GC levels play an important role in LT-mediated lethality in mice.

MATERIALS AND METHODS

Materials. PA and LF were purified as previously described (49) and prepared in sterile phosphate-buffered saline (PBS). All PA and LF preparations from *Bacillus anthracis* are endotoxin free and do not induce any tumor necrosis factor alpha in mouse macrophages. Concentrations and doses of LT refer to the amounts of each component (i.e., 100 μg LT is 100 μg PA plus 100 μg LF). Mifepristone (RU486), dexamethasone (DEX), and aldosterone (ALDO) were purchased from Sigma (St. Louis, MO).

Animals. ADX and control colony age-matched male BALB/cJ, C57BL/6J, and DBA/2J mice (8 to 12 weeks, 20 to 22 g) were transferred from Jackson Laboratories (Bar Harbor, Maine) 5 to 7 days following surgery. ADX and control mice received 0.9% saline in drinking water throughout all experiments. Saline helps adrenalectomized mice compensate for loss of mineralocorticoids that regulate salt reabsorption through renal tubules. Mice were injected intraperitoneally (i.p.) with 1.0 ml of 100 μg LT (100 μg PA plus 100 μg LF/ml in PBS) or PBS. In experiments involving multiple toxin injections, mice were injected i.p. (0.5 ml) with 25 μg LT, 50 μg LT, or PBS and reinjected 12 h or 24 h later with the appropriate toxin dose for a total of 100 μg LT/mouse over two injections. In some experiments, DEX or ALDO was provided throughout the experiment in drinking water, beginning 24 h prior to LT injection. Drug solutions were made in 100% ethanol prior to dilution in drinking water to 1% (vol/vol). Mice consistently consume 2.5 to 3.5 ml/day, resulting in very little variability in final doses of drug delivered. Alternatively, DEX was also delivered via daily i.p. injections (0.5 ml). For RU486 experiments, mice received drug (i.p., 1 ml) at 16 h prior to LT or PBS injections and daily thereafter. Four different vehicles were used for delivery of RU486 in independent experiments (0.5% methylcellulose plus 0.1% Tween 80 in PBS; 0.5% Tween 80 in PBS; vegetable oil and 2% ethanol in PBS). RU486 was given at 1.2 mg/kg of body weight, 4.0 mg/kg, and 10.0 mg/kg. In other experiments, to test different routes of LT and drug delivery, mice received 600 μg drug (30 mg/kg, i.p., 0.5 ml) or vehicle (0.5% methylcellulose plus 0.1% Tween 80 in PBS) 3 h prior to LT (50 μg, intravenous) or PBS injections. RU486 or vehicle were administered once every 24 h throughout the experiment.

Cytotoxicity assays. Peritoneal macrophages were elicited by injection of 2 ml filter-sterilized 4% Brewer modified thioglycolate (Becton Dickinson, Cockeysville, MD) in PBS and harvested 72 h later by peritoneal lavage. Cells were seeded in 96-well plates with Dulbecco's modified Eagle medium with 10% fetal calf serum, 2 mM glutamine, and 50 μg/ml gentamicin at 37°C in 5% CO_2 for 8 h prior to treatment with twofold dilutions of LT. Cell viability was assessed 150 min after LT addition using MTT dye [3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyltetrazolium bromide] (Sigma, St. Louis, MO) as previously described (49).

Cytokine expression. Interleukin 1 beta, MCP-1/JE (monocyte chemoattractant protein-1), KC, eotaxin, and tumor necrosis factor alpha levels in serum were measured by enzyme-linked immunosorbent assay (R&D systems, Minneapolis, MN) following the manufacturer's protocols.

Assay for GR function. Mice were injected with LT (100 μg) or PBS 40 min prior to i.p. DEX injection (60 μg/0.5 ml/mouse). Control mice were treated with PBS or left untreated. After 5 h, livers were homogenized in ice-cold buffer (0.2 mM pyridoxal phosphate, 0.5 mM alpha-ketoglutarate, 0.1 M potassium phosphate, pH 7.6), and centrifuged at 100,000 × *g* (4°C for 30 min), and tyrosine aminotransferase (TAT) activity was determined as described previously (44). In select experiments, mice were pretreated with 25 μg LT and DEX was injected after 24 h. Livers were harvested 5 h post-DEX injection and processed as described above.

Corticosterone (CORT) measurements. To minimize stress, all mice were redistributed at the same time (three mice/cage) 48 h prior to each experiment. Animals were housed on a 12-h light cycle. On the day of experiment, at the midpoint of the light cycle, to minimize stress, only one mouse per cage was injected with either LT (100 μg) or PBS (controls) and rehoused with cage mates. At 3 h postinjection, trunk blood was collected from experimental subjects immediately after cervical dislocation. Only a single mouse was removed from each cage. In parallel experiments, mice were euthanized by CO_2 prior to trunk blood collection. Serum samples were analyzed by Linco Diagnostic Services, Inc. (St. Charles, MO) using radioimmunoassay methods.

RESULTS

ADX mice are sensitized to LT toxicity. ADX greatly increased susceptibility of BALB/cJ (LT-susceptible), C57BL/6J (intermediate susceptibility to LT), and DBA/2J (LT-resistant) mice to a dose of 100 μg LT (Fig. 1).

LT repression of GR activity is similar in BALB/cJ, C57BL/6J, and DBA/2J mice. LT inhibits DEX-induced GR gene activation both in vitro and in vivo (56), as seen by LT repression of DEX-induced TAT gene activity. BALB/cJ mice showed consistently higher basal and PBS-induced TAT activity than DBA/2J mice (Fig. 2A), resulting in an apparent severalfold-higher induction of GR-mediated gene expression in the DBA/2J mice than in the BALB/cJ mice (Fig. 2B). The C57BL/6J mice showed a weaker response to DEX (Fig. 2A and B). LT repression of DEX-induced TAT activity (comparison of DEX induction of TAT with and without LT) was similar for all strains (55 to 70%) (Fig. 2B). These results indicate that although there are strain differences in GR responsiveness to DEX, strain differences in susceptibility to LT are not due to differences in LT repression of GR.

ADX does not alter macrophage sensitivity and cytokine response to LT in DBA/2J mice. BALB/cJ macrophages are highly LT sensitive, and mice show a transient induction of cytokines in response to toxin (31), while C57BL/6J and DBA/2J have LT-resistant macrophages and have no cytokine response (31, 32). The presence of LT-sensitive macrophages in C57BL/6J mice is sufficient to result in BALB/cJ-like susceptibility to LT challenge (32). Therefore, we investigated the effect of ADX on macrophage viability and cytokine response in DBA/2J and ADX-DBA/2J mice. Although ADX rendered DBA/2J animals highly susceptible to toxin, ADX-DBA/2J macrophages remained LT resistant (Fig. 3A) and did not show the macrophage lysis-associated cytokine response previously described (32) (Fig. 3B to F). Neither macrophage sensitivity nor cytokine production is responsible for the higher LT-associated mortality of ADX-DBA/2J mice.

LT-induced corticosterone levels differ between BALB/cJ and DBA/2J mice. Differential CORT responsiveness correlates with differential inflammatory responses in rodent strains (2, 3). Comparison of CORT responses to LT showed that BALB/cJ mice had four- to fivefold-higher plasma CORT lev-

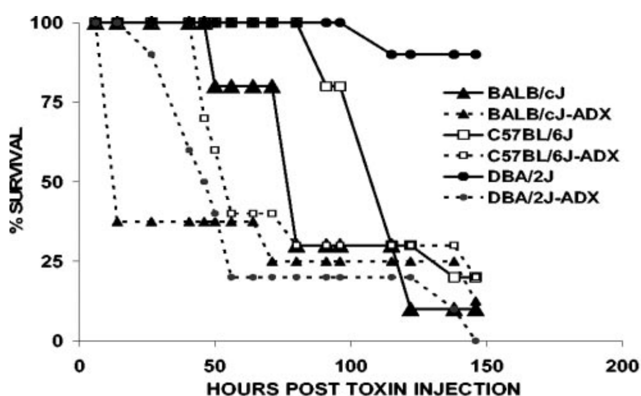

FIG. 1. Comparison of LT toxicity in normal and ADX mice. Mice were injected i.p. with 100 μg LT, and survival was monitored. Survival percentages (n = 10 for all strains) are given.

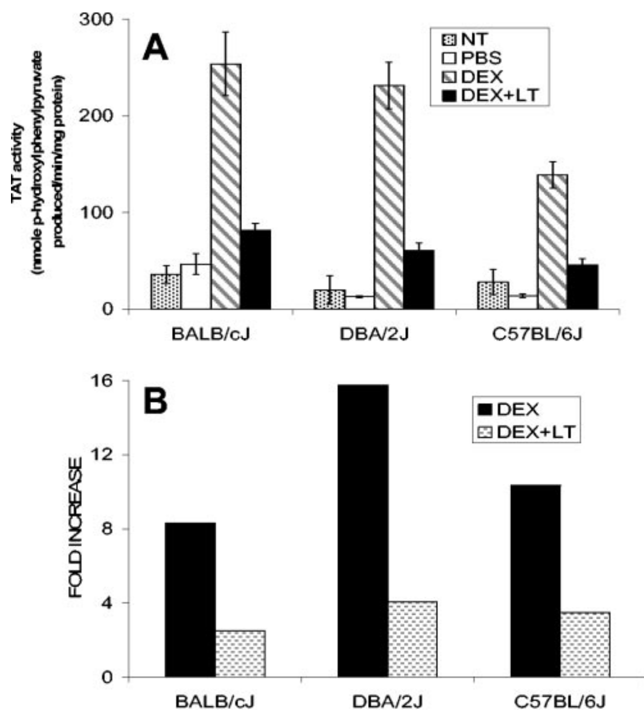

FIG. 2. LT-mediated repression of GR activity. (A) Groups of age-matched male mice were injected i.p. (1.0 ml) with LT (100 μg) followed by i.p. injection of DEX (60 μg/0.5 ml) after 40 min (DEX + LT). Positive controls were injected i.p. (1.0 ml) with PBS followed by DEX injection after 40 min (DEX). Negative controls were either injected with PBS alone (PBS) or left untreated (NT). Livers were harvested from animals 5 h after DEX injection and assayed for the GR-regulated tyrosine aminotransferase (TAT) activity. Experiments were performed with three mice/treatment/strain, except for NT groups, which contained two mice/strain/experiment. Presented are the combined averages of three independent experiments with associated standard deviation ($n = 9$ for each treatment, except for NT [$n = 6$]). (B) The induction of TAT activity by DEX was calculated by comparing the fold induction of the PBS-pretreated and LT-pretreated groups which received DEX injections to that of the negative controls receiving only PBS injections. Presented are the combined averages of three independent experiments ($n = 3$ for each treatment).

els than PBS-treated controls, while LT-treated DBA/2J mice showed no CORT induction above that in PBS controls (Fig. 4).

GR antagonist treatment sensitizes BALB/cJ but not DBA/2J mice to LT. To determine whether susceptibility of ADX mice was due to loss of endogenous GC response, we treated BALB/cJ or DBA/2J mice with the GR/progesterone antagonist RU486 prior to treatment with LT. Treatment of BALB/cJ mice with RU486 exacerbated toxicity and mortality in a manner similar that seen with ADX (data not shown). However, daily treatment of DBA/2J mice with RU486 (tested doses: 1.2, 4.0, 10, and 30 mg/kg) failed to sensitize DBA/2J mice to the lethal effects of LT beyond effects of vehicle alone (data not shown).

LT-resistant DBA/2J mice are sensitized to LT by toxin pretreatment. It is possible that RU486, a ligand-competitive GR antagonist, did not sensitize ADX-DBA/2J mice due to the lack of CORT induction by LT in these animals. A low dose of LT, a noncompetitive ligand-independent inhibitor of GR activity (56), was used to block GR activity, followed after 12 h or 24 h by a second normally nonlethal dose of LT. Previous studies have indicated that levels of available PA receptor in rats treated with presumed saturating doses of toxin have returned to normal by 24 h (33). A single 100-μg LT dose is not lethal to DBA/2J mice (32); therefore, this dose was not exceeded in the combined treatments. Groups of mice were treated with either 25 μg or 50 μg of toxin, followed by 75 μg or 50 μg, respectively, at either 12 h or 24 h. The only treatment that resulted in 100% mortality in two independent experiments was the 25-μg pretreatment with LT, followed by 75 μg at 24 h (Fig. 5). Approximately half the mice died with the 50-μg pretreatment followed by 50 μg at 24 h (Fig. 5). In groups where the second toxin treatment was administered at 12 h, mortality was lower, possibly due to reduced numbers of PA receptors (33). LT-mediated repression of GR activity at 25 μg LT over a 24-h period was found to be at least as potent as the 100-μg dose in inhibition of DEX-induced GR transactivation (Fig. 5, inset). It is possible that this sensitization of DBA/2J mice by low-dose LT pretreatment occurs by mechanisms completely independent of GR repression. However, it is also possible that this sensitization represents events similar to those occurring in ADX-mediated sensitization.

GC treatment did not rescue ADX mice but sensitized intact DBA/2J and BALB/cJ mice to LT. Increased susceptibility to various insults in ADX animals can, in many cases, be reversed with GC replacement therapy (54). Oral DEX treatment (300 μg/ml, 100 μg/ml, or 10 μg/ml DEX in 0.9% saline) did not rescue ADX-DBA/2J from LT toxicity, but surprisingly, it sensitized adrenally intact DBA/2J mice to LT (Fig. 6A). The i.p. delivery of DEX produced similar results (data not shown).

BALB/cJ mice have a large but transient cytokine response to LT which can contribute to toxicity (32). We tested whether GC inhibition of this response could provide some protection from LT. Contrary to this prediction, oral DEX treatment exacerbated LT lethality in adrenally intact BALB/cJ mice, resulting in a more rapid appearance of malaise and subsequent mortality in a manner similar to that with ADX or with RU486 treatment of this strain (Fig. 6B).

Oral aldosterone therapy does not rescue adrenalectomized mice from LT lethality. We tested whether the loss of the adrenal hormone ALDO, an important regulator of cardiovascular function (9, 20, 46), affected LT susceptibility. ALDO replacement therapy, similarly to GC therapy, was not successful in rescuing sensitized ADX-DBA/2J mice from LT (data not shown). Treatment with a combination of ALDO and DEX also was not successful in rescuing ADX animals from LT (data not shown). Therefore, ALDO and GC therapy alone are not sufficient to reverse sensitization of ADX animals, and altering the basal levels of these hormones in intact, adrenally sufficient mice can increase susceptibility to LT lethality.

DISCUSSION

We report here that manipulation of GC levels in mice, through either ADX or DEX therapy, renders normally LT-resistant mice susceptible to the lethal effects of this toxin. This is the first report showing reversal of LT resistance in the resistant DBA/2J strain.

We have previously shown that LT selectively represses GR transactivation through non-ligand-binding, noncompetitive

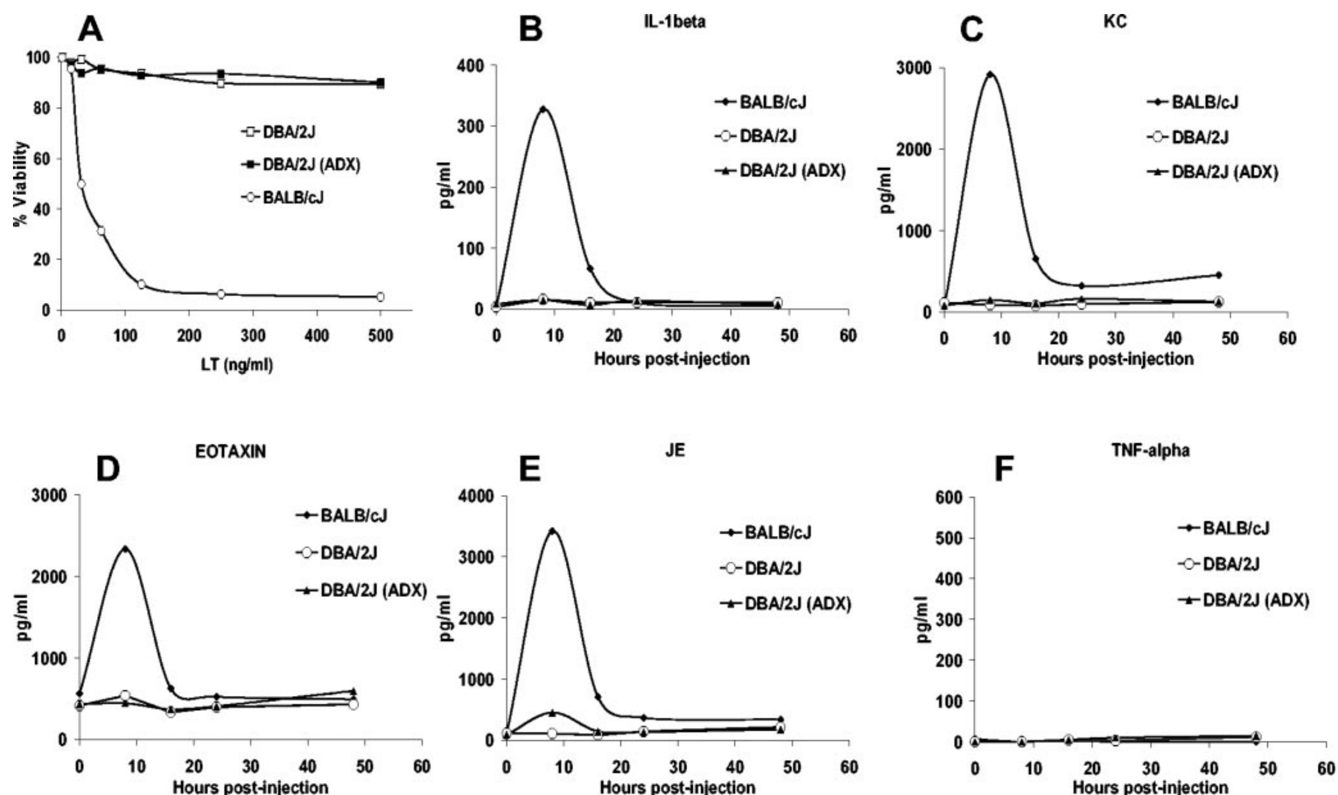

FIG. 3. Macrophage sensitivity and cytokine response to LT in intact versus ADX mice. (A) Viability of LT-treated peritoneal macrophages from DBA/2J, ADX-DBA/2J, and BALB/cJ. (B-F) Levels of cytokines in sera from BALB/cJ, DBA/2J, and ADX-DBA/2J mice after injection of LT (100 μg). Averages are presented from three individual mice, per strain, per time point.

mechanisms (56). We postulated that if LT's repression of GR transactivation plays a role in LT-induced mortality, then interruptions of the GC response by other means (ADX or RU486) should enhance mortality in otherwise resistant strains (DBA/2J). Since LT repression of the GR occurs at a step downstream of ligand binding and is noncompetitive, we postulated that while GC treatment might reduce LT mortality in ADX animals, it would be unlikely to prevent mortality in the context of inactivated GR. Our findings in part support these hypotheses and in part are counter to them.

We found that ADX did render otherwise LT-resistant DBA/2J mice highly susceptible to LT mortality and also enhanced LT mortality in already susceptible BALB/cJ and C57BL/6J strains. However, there was no difference in LT-mediated GR repression between strains, indicating that this inhibitory function did not appear to explain differential susceptibilities to toxin.

We also observed strain differences in CORT responsiveness to LT exposure, with susceptible BALB/cJ mice showing a high CORT response, while resistant DBA/2J animals showed no CORT response to toxin. In this context, LT appears to stimulate the HPA axis in BALB/cJ mice, as do other bacterial products (24, 30, 55). Such strain differences in HPA axis responses are associated with differential susceptibilities to autoimmune/inflammatory diseases, with high CORT responses correlating to resistance (24, 30, 55), often through the anti-inflammatory effects of GCs (10, 24, 36, 45, 54).

In cases where mortality after exposure to infectious agents is related to proinflammatory host responses, interruption of the GC response with concomitant loss of GC anti-inflammatory effects renders otherwise hyper-GC-responsive resistant hosts susceptible to rapid mortality. Reconstitution of the HPA axis reverses this effect (40, 54). However, inflammatory responses to LT are not a major determinant in LT lethality in mice (31, 32). In fact, hyper-CORT-responsive BALB/cJ mice are LT sensitive, while hypo-CORT-responsive DBA/2J mice are resistant. Our findings that DEX treatment enhanced LT mortality in both BALB/cJ mice and DBA/2J mice support the idea that sensitivity to the lethal effects of LT may be associated with high GC levels.

BALB/cJ mice also have a rapid but transient cytokine response with a potent interleukin 1 beta release associated with LT treatment due to lysis of their LT-sensitive macrophages. This response is absent in C57BL/6J and DBA/2J mice (31, 32). While not the cause of LT-induced mortality, this cytokine response can contribute to exacerbation of toxicity (31, 32). This cytokine burst could trigger the high CORT induction seen in BALB/cJ mice, which in turn would lead to the subsequent observed rapid shutdown of the cytokine response (31, 32). The observation that RU486 treatment, shutting down the GC response that would inhibit the cytokine responses, does in fact exacerbate LT mortality in high-CORT, high-cytokine-responsive BALB/cJ mice supports a role for cytokine release contributing to further LT toxicity in this strain. Additional

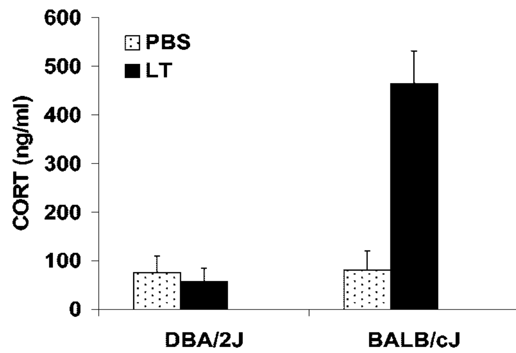

FIG. 4. CORT induction by LT. CORT levels in sera collected 3 h after LT (100 μg) or PBS injection. Results were similar for both cervical dislocation and CO_2 euthanasia methods and are presented as combined averages with standard deviations based on 10 (DBA/2J) and 12 (BALB/cJ) mice for each treatment.

genetic factors also certainly play a role in susceptibility to LT. Our finding that ADX in normally LT-resistant DBA/2J mice has no effect on macrophage sensitivity to LT or cytokine production, while greatly enhancing mortality, supports this (31, 32). In fact, two LT susceptibility loci in addition to the macrophage susceptibility locus have been found in the DBA/2J strain (29).

The lack of ability of RU486 to sensitize DBA/2J mice to LT mortality beyond the level with vehicle is consistent with the lack of LT induction of CORT in this strain, since RU486 competes with the natural ligand (CORT) for binding to GR, and in the context of low CORT would have little effect. The data showing that LT mortality was significantly enhanced by pretreatment with sublethal doses of LT cannot be directly linked to GR function, since sensitization could be due to other effects of LT. However, these low doses of LT that noncompetitively block GR function may indicate that an optimal level of functional GRs is needed to protect against LT mortality. A second exposure to sublethal doses of LT in this context, causing sensitization similar to ADX, could possibly be due to the first dose of LT acting as a GR antagonist to block basal GR activity prior to secondary LT exposure. Investigation of the actual mechanism for sensitization of DBA/2J mice through low-dose LT treatment requires further study.

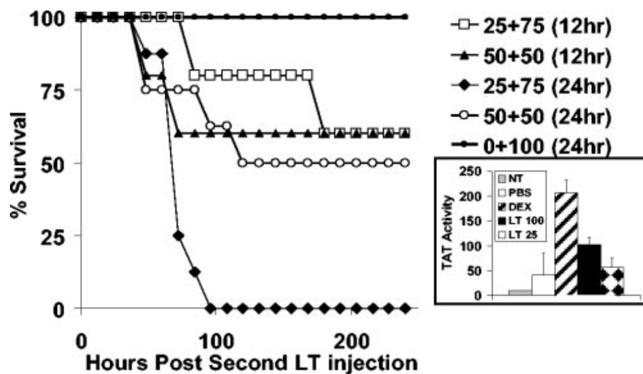

FIG. 5. Sensitization of DBA/2J mice to toxin by multiple toxin treatments. Survival rates are shown for DBA/2J mice injected with 0 μg (i.e., PBS alone), 25 μg, or 50 μg LT, followed by injection with a second dose of toxin at 12 h or 24 h, for a total combined injected dose of 100 μg LT. Results are based on the following mouse numbers for each treatment: 12-h data, $n = 5$; 24-h data, $n = 8$; PBS only, $n = 4$. LT-mediated GR repression was verified at the 25-μg LT dose over 24 h (inset). Mice were injected i.p. with 100 μg or 25 μg of LT (LT 100 and LT 25), followed by i.p. injection of DEX (60 μg/0.5 ml) after 24 h ($n = 3$). Positive controls were injected i.p. (1.0 ml) with PBS, followed by DEX injection after 24 h (DEX). Negative controls were either injected with PBS alone (PBS) or left untreated (NT). TAT activity was measured 5 h after DEX injection.

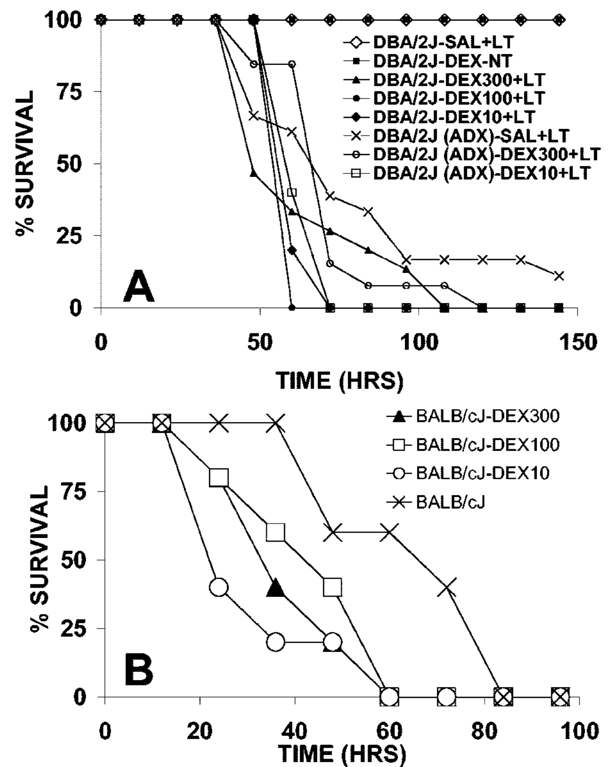

FIG. 6. Oral GC therapy for LT-treated mice. (A) DBA/2J control or adrenalectomized [DBA/2J (ADX)] mice were supplied with saline as drinking water alone [DBA/2J-SAL and DBA/2J (ADX)-SAL groups] or supplemented with 300, 100, or 10 μg/ml DEX starting 24 h prior to LT (100 μg) injection and throughout the experiment. A control group received DEX in drinking water but no toxin treatment (DBA/2J-DEX-NT). Survival was monitored after toxin injection. Results are based on the following mouse numbers: DBA/2J-SAL+LT, $n = 10$; DBA/2J-DEX-NT, $n = 5$; DBA/2J-DEX300+LT, $n = 15$; DBA/2J-DEX100+LT, $n = 5$; DBA/2J-DEX10+LT, $n = 5$; DBA.2J (ADX)-SAL+LT, $n = 18$; DBA/2J (ADX)-DEX300+LT, $n = 13$; DBA/2J (ADX)-DEX10+LT, $n = 5$. (B) Similar experiments were performed with normal BALB/cJ mice provided with water (controls) or three different doses of DEX (10 μg/ml, 100 μg/ml, and 300 μg/ml) in drinking water 24 h prior to LT injection (100 μg) and continuing throughout the experiment. Results are based on five mice per group.

The finding that DEX treatment did not reverse or prevent mortality in ADX BALB/cJ mice in the presence of LT, a noncompetitive GR repressor, is consistent with LT inactivation of the receptor downstream of ligand binding. However, DEX enhancement of mortality suggests that interruption of the HPA axis by ADX and augmentation with DEX enhance mortality through different and independent mechanisms.

Exogenous DEX treatment could reduce the number or activity of GRs through negative feedback. Alternatively, DEX

could exacerbate LT-mediated shutdown of MEK pathways through inhibition of p38 and other mitogen-activated protein kinase pathways, although the link between MEK shutdown and LT mortality is currently not known (10, 27). Removal of protective (antiapoptotic) mechanisms through transrepression of NF-κB and AP-1 by DEX may also lead to a more-rapid death from the sequelae of MEK cleavage or other LT-mediated events (34). Finally, GCs could affect vascular homeostasis and blood pressure and the outcome of LT-mediated vascular collapse through regulation of the epithelial sodium channel (ENaC) and other factors (5, 28, 47, 58) or through control of hypoxic responses (5, 23, 26, 39, 52). Regardless of the mechanism, our findings show that excess GCs increase LT mortality. Indeed, it has been found with human patients that high doses and prolonged treatment of septic shock with GCs is detrimental to survival, and increased cortisol levels have been found in critically ill patients (13, 21, 43, 50).

ADX enhancement of mortality in both mouse strains suggests that GCs are not essential for LT-mediated lethality. ADX sensitization to LT-mediated vascular collapse may be related to loss of other adrenal factors, including vascular mediators, such as aldosterone, epinephrine and norepinephrine, that are essential to maintaining blood pressure in shock states (31). Failure of ALDO replacement in reversing LT mortality suggests that additional factors are also involved.

In summary, we report here that both ADX and DEX treatment significantly enhance LT-induced mortality, likely by different mechanisms that remain to be determined. Clearly an optimal GC response is required to adequately protect against LT mortality. The finely tuned HPA response, easily shifted by many stimuli, including stress and infection, can alter susceptibility to LT. Thus, experiments carried out in different animal facilities with the same strain of mice may show strikingly different susceptibilities to LT, based on the previous or current activation state of the HPA axis. In fact, LT doses used in current studies to kill rodents (14, 31) are often significantly higher than those reported in the 1980s (17, 57). This difference may be due to differential prior infection status rather than to toxin preparations, particularly since mice in the 1980s often had subclinical infections with agents that are now routinely diagnosed and eliminated from animal facilities. In these reports the toxin used in both old and new studies was prepared by the same protocol in the same lab, and testing these preparations in macrophage and mouse toxicity assays revealed no difference in potency (data not shown). Thus, in studies of anthrax and LT effects, especially when analyzing "resistant" and "sensitive" animals, it is crucial to be aware of the HPA activation state and previous stress exposure of experimental subjects. Accordingly, the findings presented in this study, if applicable to humans, have important implications for the role of host neuroendocrine responsiveness and treatments targeting these hormones in the course and outcome of LT-mediated shock.

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Editor: J. D. Clements