

Endogenous and Exogenous Glucocorticoids in Experimental Enterococcal Infection

Christopher J. Papasian,* Nilofer Qureshi, and David C. Morrison

Department of Basic Medical Science and Shock Trauma Research Center, University of Missouri—Kansas City, School of Medicine, Kansas City, Missouri 64108

Received 4 November 2005/Returned for modification 12 December 2005/Accepted 19 December 2005

The potentially protective role of the host adrenal-glucocorticoid response to enterococcal infection was evaluated in an experimental model in which mice were infected intraperitoneally with two distinct *Enterococcus faecalis* strains (K9 and CP-1). We demonstrated that corticosterone levels in serum and peritoneal-lavage fluid were elevated within 1 hour of infection with either *E. faecalis* strain. We also demonstrated that adrenalectomized mice generated a more robust localized peritoneal tumor necrosis factor alpha (TNF- α) response to both *E. faecalis* strains than did sham-adrenalectomized mice but that neither *E. faecalis* strain induced a systemic TNF- α response. Further, peritoneal TNF- α production in adrenalectomized mice infected with either *E. faecalis* K9 or CP-1 was suppressed by prior treatment with an exogenous glucocorticoid (dexamethasone). The potential clinical significance of these results was suggested by our findings that adrenalectomy markedly increased susceptibility (a >100-fold decrease in the 50% lethal dose) to lethal infections with *E. faecalis* CP-1 and that prior dexamethasone treatment partially compensated for adrenalectomy. In marked contrast to these findings, however, adrenalectomy did not substantially increase susceptibility to lethal *E. faecalis* K9 infection. Further, preinfection with *E. faecalis* CP-1 1 hour before infection with *E. faecalis* K9 did not protect mice from lethal *E. faecalis* K9 infections. Collectively, these studies indicate that the host can generate a glucocorticoid response to *E. faecalis* infection that suppresses TNF- α production. Further, this glucocorticoid response can protect the host from potentially lethal *E. faecalis* infections, but different strains show heterogeneity with respect to the extent of protection afforded by the adrenal-glucocorticoid response.

The pathogenesis of microbial sepsis is generally recognized to involve the systemic production of a diverse array of inflammatory cytokines in response to microbes or microbial products (5, 13). This inflammatory cascade can become self-sustaining when cytokines produced early in the infectious process (e.g., tumor necrosis factor alpha [TNF- α] and interleukin 1 β [IL-1 β]), induce further production of these and other proinflammatory cytokines (13, 35). Inflammatory cytokines, such as IL-1 β and IL-6, also activate the adrenal glands, resulting in rapid increases in plasma glucocorticoid levels, which help modulate the inflammatory response by suppressing further production of proinflammatory cytokines and by regulating the circulatory response (4, 15, 32, 34, 36, 37). The contribution of endogenous glucocorticoids to the host's defense against infection has been highlighted by studies with adrenalectomized animals. In those studies, markedly increased mortality was found in adrenalectomized animals after injection with *Escherichia coli*, bacterial lipopolysaccharide, IL-1 β , or TNF- α ; this increased mortality was essentially eliminated by administering exogenous glucocorticoids to adrenalectomized animals prior to challenge (3, 6, 12, 16).

The results from several clinical trials have demonstrated that low-dose hydrocortisone regimens improve outcomes in sepsis, supporting the concept that adrenal insufficiency contributes to mortality in sepsis (2, 8, 21, 22). The relationship between adrenal insufficiency and sepsis in humans, however,

is complex. Critically ill patients are at increased risk for developing sepsis, the overall incidence of adrenal insufficiency in critically ill patients approximates 30%, and adrenal insufficiency is particularly common in septic patients (7, 20, 27). It has also been shown, however, that sepsis can lead to adrenal insufficiency (17, 30, 38). Consequently, the high rate of adrenal insufficiency in septic patients may be explained, at least in part, by the capacity of septic infections to induce adrenal insufficiency. It is also tempting to speculate, however, that preexisting adrenal insufficiency in a critically ill patient would significantly increase the likelihood that any subsequent infection with an opportunistic pathogen will progress to sepsis.

Enterococcus spp. are consummate opportunistic pathogens, commonly identified as causative factors in sepsis. They are considered comparatively avirulent gram-positive bacteria, as indicated by their relative inability to invade intact tissue, their relatively high 50% lethal doses (LD₅₀s) in experimental animal models of infection, and the rarity with which they produce infections in individuals without severe underlying illness (14, 23, 25). In critically ill hospitalized patients, however, enterococci frequently produce severe infections, often leading to sepsis and death (10, 19, 25, 28). In a recent study of sepsis syndrome at eight academic medical centers, *Enterococcus* spp. caused 6.1% of the total of 866 cases evaluated (28). Additionally, the mortality attributable to enterococcal bacteremia has been estimated by comparing the mortality of patients with enterococcal bacteremia to that of cohorts of nonbacteremic patients whose underlying illness resembled that of patients with enterococcal bacteremia (10, 19). In the first study, which investigated bacteremia due to vancomycin-susceptible enterococci (VSE), 43% of patients with VSE bacteremia died and

* Corresponding author. Mailing address: Dept. of Basic Medical Science M3-C03, UMKC School of Medicine, 2411 Holmes St., Kansas City, MO 64108. Phone: (816) 235-2299. Fax: (816) 235-6517. E-mail: papasianc@umkc.edu.

12% of controls died, so the mortality directly attributable to VSE bacteremia was 31%. In a more recent study, exploring bacteremia due to vancomycin-resistant enterococci (VRE), 67% of bacteremic patients and 30% of controls died, so the mortality attributable to VRE bacteremia was 37%. In the latter study, more than 80% of patients with VRE bacteremia progressed to severe sepsis and septic shock (10). Collectively, these studies underscore the importance of enterococci as common causes of serious infections in critically ill, hospitalized patients and the significant potential for these infections to progress to severe sepsis, septic shock, and death.

Surprisingly, the host response leading to septic enterococcal infections has not been well characterized. We recently reported that mice infected with *Enterococcus faecalis* generate a profile of cytokine responses that differs markedly from that described for lipopolysaccharide, gram-negative bacteria, and most gram-positive bacteria (25). Most significantly, lethal enterococcal infection failed to induce a detectable systemic TNF- α response and induced only a muted, localized TNF- α response within the peritoneal cavity, suggesting that the acute fatality of *E. faecalis* infections may well occur by a TNF- α -independent mechanism. The finding that *E. faecalis* infection did induce a rapid systemic IL-6 response, which in other experimental models has been demonstrated to stimulate a protective adrenal response, led us to explore the role of the adrenal response in the pathogenesis of enterococcal infections.

The experiments presented here support the concept that intraperitoneal (i.p.) *E. faecalis* infection induces an endogenous adrenal-glucocorticoid response that serves to suppress local TNF- α production within the peritoneal cavity. Circulating TNF- α , however, remained undetectable in adrenalectomized *E. faecalis*-infected mice, indicating that the failure of *E. faecalis* to induce a systemic TNF- α response was not due to glucocorticoid-mediated suppression of TNF- α production. Further, adrenalectomy markedly increased the susceptibility of mice to lethal infection with only one of the two strains of *E. faecalis* examined.

MATERIALS AND METHODS

Bacterial isolates. *E. coli* O111:B4 was obtained from List Biological Laboratories (Campbell, CA). *E. faecalis* isolate CP-1 was a clinical isolate from the collections of the Truman Medical Center (Kansas City, MO). *E. faecalis* K9 was a generous gift from Rebecca Horvat from the collection of clinical isolates at the University of Kansas Medical Center (Kansas City, KS). Preliminary identification of enterococcal isolates was based on colony and Gram stain morphology, production of pyrrolidonyl arylamidase, and absence of catalase production. Subsequent identification to the species level was accomplished with a Positive Combo Panel (Dade International Microscan, Inc., Sacramento, CA).

Several colonies from a streaked plate grown overnight on Trypticase soy agar were used to initiate bacterial growth. Bacteria were inoculated into Trypticase soy broth and grown overnight at 37°C with aeration. The following morning, 0.1 ml of this culture was transferred to 50 ml of fresh medium and the bacteria were grown to mid-log phase. The bacteria were then washed three times in sterile saline and diluted either in sterile saline for *in vivo* cytokine stimulation or in phosphate-buffered saline (PBS) for sepsis lethality studies. For long-term storage, organisms were frozen at -70°C in tryptic soy broth with 15% glycerol (Remel, Lenexa, KS).

Experimental animals. Outbred CF-1 female mice, including adrenalectomized and sham-adrenalectomized mice, 6 to 8 weeks of age, were purchased from Charles River Laboratories, Inc. (Wilmington, MA). All mice were monitored at the University of Missouri—Kansas City American Association for Assessment and Accreditation of Laboratory Animal Care-accredited animal facility for 5 to 10 days before being used for experiments, with food and water or saline (adrenalectomized mice only) provided *ad libitum*. Experiments with

adrenalectomized and sham-adrenalectomized mice were conducted a minimum of 13 days after the animals had undergone surgery.

Reagent. Dexamethasone was purchased from American Regent Labs (Shirley, NY).

TNF- α assays and corticosterone assays. TNF- α concentrations in serum and peritoneal-lavage fluid were determined using the Quantikine M Mouse TNF- α Immunoassay kit (R&D Systems, Minneapolis, MN). The minimum concentration of TNF- α stated by the manufacturer to be detectable by this assay is 5.1 pg/ml. Corticosterone concentrations in serum and peritoneal-lavage fluid were determined using the Corticosterone Immunoassay kit (R&D Systems, Minneapolis, MN). The manufacturer's protocols for both TNF- α and corticosterone assays were followed exactly. All data for TNF- α and corticosterone represent the averages of duplicate samples for each specimen.

Serum and peritoneal TNF- α and corticosterone quantitation. For the analysis of cytokines in serum and peritoneal-lavage fluids, mice were injected i.p. with viable bacteria in 0.2 ml of sterile saline and were sacrificed and bled by decapitation using a small-animal guillotine. Serum was separated from clotted blood components and frozen at -70°C until it was tested. Due to small volumes, all serum specimens were minimally diluted twofold prior to being tested. Peritoneal lavage was performed immediately after sacrifice by injecting 5.0 ml of PBS into the peritoneal cavity, vigorously agitating the peritoneal cavity, reaspirating the peritoneal-lavage fluid, and freezing it at -70°C until it was tested. Peritoneal TNF- α and corticosterone levels are expressed as pg/peritoneum and ng/peritoneum, respectively. These values were determined by measuring the cytokine concentration within the peritoneal-lavage fluid and then correcting for the total volume of lavage fluid injected per mouse.

Sepsis lethality studies. Mice were injected i.p. with 0.2 ml of graded doses of bacterial suspension/mouse. In some experiments, 0.2 ml PBS alone or 100 μ g dexamethasone in 0.2 ml PBS was also injected i.p. immediately before bacterial challenge. Lethality was monitored at defined time intervals for up to 48 h. Determinations of LD₅₀s were performed according to the method of Reed and Muench at the 48-h time point (26).

Statistics. TNF- α and corticosterone levels in serum and peritoneal fluid are presented as means \pm standard errors of the mean (SEM), with differences between groups assessed for significance by the Student paired *t* test method. A calculated *P* value of <0.05 was considered significant.

RESULTS

Host corticosterone response to *E. faecalis* infection. We first addressed the question of the extent to which *E. faecalis* would elicit either a systemic or a localized corticosterone response. To do this, mice were inoculated i.p. with 5×10^9 bacteria, and at various times postinfection, blood and peritoneal-lavage specimens were obtained. As shown by the data in Fig. 1, top, serum corticosterone levels were elevated within 1 hour of i.p. infection with either of two distinct strains of *E. faecalis*. Serum corticosterone peaked at 3 h and then dropped toward baseline levels over the next several hours. Corticosterone was also measured in the peritoneal cavities of *E. faecalis*-infected mice, as shown in Fig. 1, bottom. Corticosterone was essentially undetectable in the peritoneal fluid of vehicle-challenged controls. Within 1 h of infection with either strain of *E. faecalis*, corticosterone was detected within the peritoneal cavity, and peak levels were again achieved at 3 h after infection. The corticosterone response to i.p. *E. coli* infection differed significantly relative to the response to either strain of *E. faecalis* in that serum and peritoneal corticosterone levels increased continually between 1 and 7 h after infection (data not shown).

Effect of adrenalectomy on the peritoneal and systemic TNF- α responses to *E. faecalis* infection. Next, we examined the extent to which the host adrenal response suppresses local and systemic TNF- α production in response to *E. faecalis* infection. To do this, sham-adrenalectomized and adrenalectomized mice were inoculated i.p. with 2×10^8 bacteria, and at various times postinfection, blood and peritoneal-lavage specimens were obtained. The data in Fig. 2 reveal that localized TNF- α

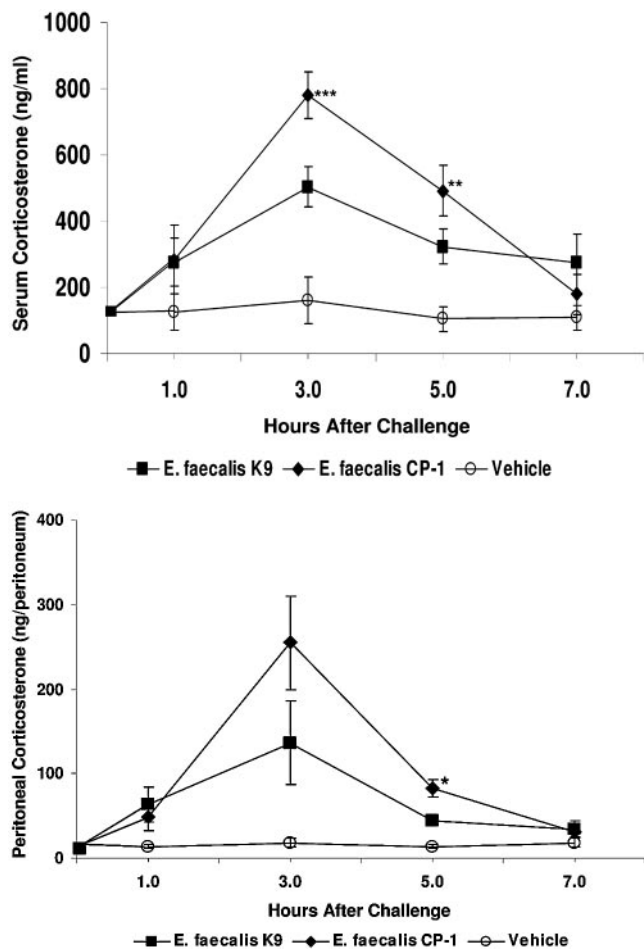


FIG. 1. Serum and peritoneal corticosterone levels following infection with *E. faecalis*. Shown are serum (top) and peritoneal corticosterone levels of CF-1 mice injected i.p. with *E. faecalis* CP-1 or K9 (5×10^9 CFU/mouse) or vehicle. Each data point represents the mean \pm SEM for five female outbred mice. Statistical significance of differences between corticosterone levels detected in *E. faecalis* CP-1-versus K9-infected mice: ***, $P < 0.001$; **, $P < 0.05$; *, $P < 0.01$.

production in response to infection with two distinct strains of *E. faecalis* is significantly enhanced in adrenalectomized mice compared to sham-adrenalectomized controls. At their peak, 3 h after infection with *E. faecalis* K9, peritoneal TNF- α levels were more than 10-fold higher in adrenalectomized mice than in sham-adrenalectomized controls (Fig. 2, top). Similar results were attained with *E. faecalis* CP-1; 3 to 5 h after infection, peritoneal TNF- α levels in adrenalectomized mice were five to sevenfold higher than TNF- α levels in sham-adrenalectomized controls (Fig. 2, bottom). In contrast to the localized peritoneal TNF- α response, adrenalectomy did not lead to the development of a systemic TNF- α response to *E. faecalis* infection; TNF- α was not detected (<10 pg/ml) in the sera of either adrenalectomized or sham-adrenalectomized mice 1, 3, 5, and 7 h after i.p. infection with either strain of *E. faecalis* (data not shown). The bacterial inocula for this experiment and the experiment presented in Fig. 3 are reduced compared to the inocula used in the experiments summarized in Fig. 1, because we wanted to maximize the host response but were

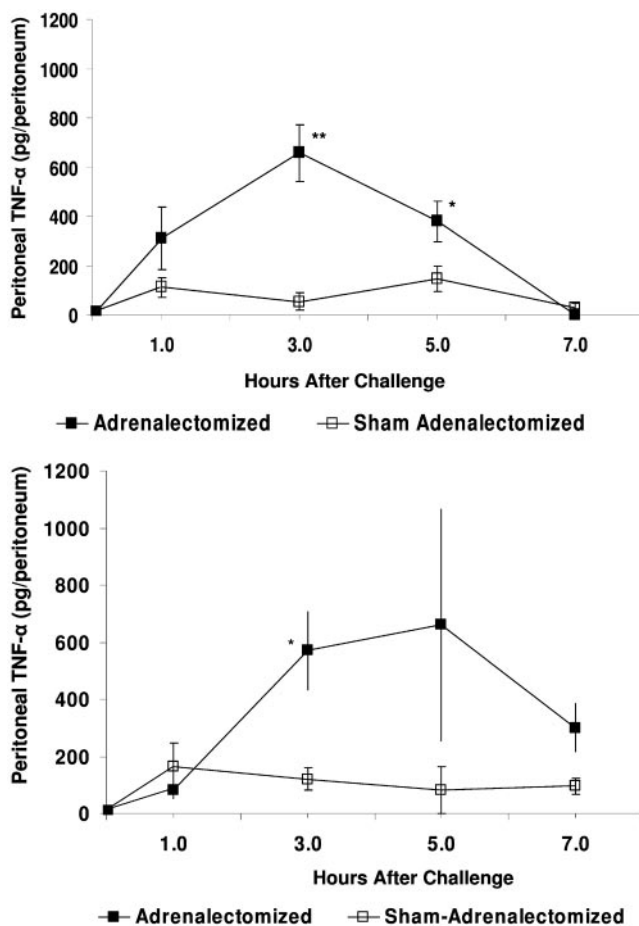


FIG. 2. Effects of adrenalectomy on peritoneal TNF- α levels in *E. faecalis*-infected mice. Shown are peritoneal TNF- α levels in adrenalectomized and sham-adrenalectomized mice injected i.p. with *E. faecalis* K9 (top) or *E. faecalis* CP-1 (bottom) (2×10^8 CFU/mouse). Each data point represents the mean (\pm SEM) TNF- α levels for five female outbred CF-1 mice. **, $P < 0.005$; *, $P < 0.05$.

concerned that adrenalectomized mice would die if given a higher inoculum.

Exogenous glucocorticoid administration suppresses the TNF- α response of adrenalectomized mice. The experiments presented above demonstrate that TNF- α production in response to *E. faecalis* infection is markedly enhanced in mice lacking adrenal glands, suggesting that TNF- α production is under strict glucocorticoid control. To further explore this concept, adrenalectomized mice were either vehicle treated or treated with dexamethasone 30 min prior to *E. faecalis* infection to determine the extent to which an exogenously administered glucocorticoid would compensate for deficient endogenous glucocorticoid production in these mice. The dexamethasone doses used in these experiments have been shown to maximally suppress host TNF- α production and to be maximally protective against lethal challenge in this experimental mouse model (12, 25, 29). As shown by the data in Fig. 3, dexamethasone treatment significantly reduced the peritoneal TNF- α response of adrenalectomized mice to infection with both *E. faecalis* strains. Collectively, these experiments lend substantial support to the

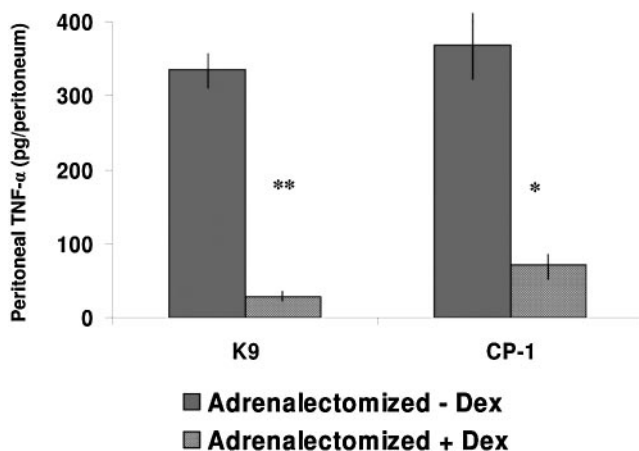


FIG. 3. Effect of exogenous glucocorticoid administration on peritoneal TNF- α levels in adrenalectomized *E. faecalis*-infected mice. Shown are peritoneal TNF- α levels in adrenalectomized mice pretreated with dexamethasone (Dex) (100 μ g i.p. in 0.2 ml PBS) or vehicle 30 min prior to i.p. injection with *E. faecalis* K9 or CP-1 (2×10^8 CFU/mouse). Each bar represents the mean (\pm SEM) TNF- α levels for five female outbred CF-1 mice. **, $P < 0.001$; *, $P < 0.01$.

concept that an adrenal-glucocorticoid response to *E. faecalis* infection markedly suppresses localized TNF- α production.

Effects of endogenous and exogenous glucocorticoids on susceptibility to lethal *E. faecalis* infection. We next explored the relationship between adrenal-gland-mediated suppression of host inflammatory responses and susceptibility of mice to potentially lethal *E. faecalis* infection. For these studies, the LD₅₀s for both *E. faecalis* strains were determined in sham-adrenalectomized controls and adrenalectomized mice pretreated with either dexamethasone or vehicle, as described in Materials and Methods. As anticipated, the LD₅₀ for *E. faecalis* CP-1 was markedly decreased in adrenalectomized mice relative to the sham-adrenalectomized controls (more than 100-fold), suggesting that glucocorticoid regulation of local inflammation may be involved in host protection against lethality (Fig. 4). Further, pretreatment with dexamethasone significantly reversed the sensitivity of these adrenalectomized mice to *E. faecalis* CP-1 infection (an approximately 10-fold increase in host resistance). Collectively, these findings indicate that the increased susceptibility of adrenalectomized mice to lethal *E. faecalis* CP-1 infection can be partially reversed by pretreatment with exogenous glucocorticoids, but not to the level of the sham-adrenalectomized controls.

In striking contrast to these findings, however, the LD₅₀ for *E. faecalis* K9 was only marginally decreased in adrenalectomized mice relative to sham-adrenalectomized controls (approximately threefold) (Fig. 4). Further, dexamethasone pretreatment essentially completely reversed the modest decrease in LD₅₀ brought about by adrenalectomy. These findings support the conclusion that adrenal regulatory control of the local host inflammatory response is not a major factor dictating overall lethality with this strain of *E. faecalis*.

The finding that the adrenal response serves to protect mice against potentially lethal *E. faecalis* CP-1, but not *E. faecalis* K9, infections is intriguing. In order to further explore this differentially protective adrenal effect, we questioned whether

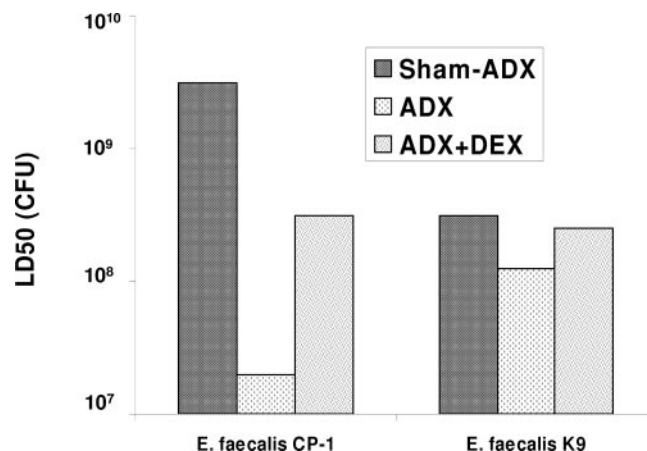


FIG. 4. LD₅₀s for sham-adrenalectomized, adrenalectomized, and dexamethasone-treated adrenalectomized mice infected with *E. faecalis*. Sham-adrenalectomized mice were treated with vehicle (Sham-ADX), and adrenalectomized mice were treated with either vehicle (ADX) or dexamethasone (ADX+DEX; 100 μ g i.p. in 0.2 ml PBS) 30 min prior to i.p. injection with graded doses of either *E. faecalis* CP-1 or K9. The *E. faecalis* inocula required to kill 50% of the mice in each group were calculated according to the method of Reed and Muench (26). Each bar represents a combination of two experiments utilizing at least 40 mice.

prior exposure to sublethal *E. faecalis* CP-1 infections might protect mice with normal adrenal function from potentially lethal *E. faecalis* K9 infections. Our results, presented in Fig. 1, suggest that the *E. faecalis* CP-1 strain elicits a significantly greater corticosterone response than does *E. faecalis* K9. As shown in Fig. 5, however, i.p. inoculation of either 10⁶ or 10⁷ CFU *E. faecalis* CP-1 1 hour prior to *E. faecalis* K9 infection had no detectable effect on the LD₅₀ of *E. faecalis* K9. These findings provide further support for the conclusion that there is

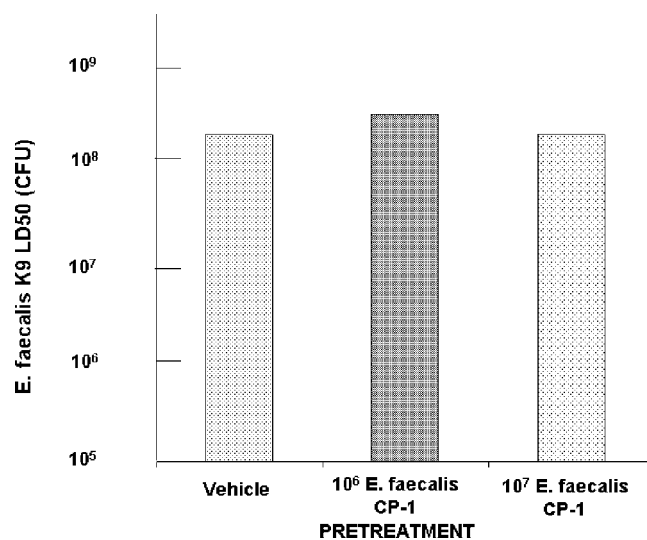


FIG. 5. *E. faecalis* K9 LD₅₀s for mice preinjected with vehicle or *E. faecalis* CP-1. Mice were injected with either vehicle, 10⁶ *E. faecalis* CP-1 organisms, or 10⁷ *E. faecalis* CP-1 organisms 1 h prior to infection with graded doses of *E. faecalis* K9. The *E. faecalis* inocula required to kill 50% of the mice in each group were calculated according to the method of Reed and Muench (26). Each bar represents data collected for 40 mice.

a relative lack of adrenal control of the overall host response to potentially lethal *E. faecalis* K9 infection, in spite of its ability to regulate localized inflammatory responses.

DISCUSSION

The experiments presented here were designed, in part, to determine the extent to which an adrenal-glucocorticoid response to *E. faecalis* infection regulates TNF- α production that in turn might influence mortality. First, we have demonstrated that i.p. infection with two distinct strains of *E. faecalis* was accompanied by time-dependent increases in corticosterone levels both in the circulation and within the peritoneal cavity. Second, we have shown that localized TNF- α production within the peritoneal cavity was markedly increased in adrenalectomized *E. faecalis*-infected mice compared to sham-adrenalectomized controls, although circulating TNF- α remained undetectable in both adrenalectomized and sham-adrenalectomized mice up to 7 h post-*E. faecalis* infection. Third, we found that the localized TNF- α response of adrenalectomized, *E. faecalis*-infected mice was suppressed by pretreatment with the synthetic glucocorticoid dexamethasone. Collectively, these data lend substantial support to the concept that in vivo TNF- α production in response to an i.p. *E. faecalis* inoculum is suppressed by an adrenal-glucocorticoid response.

Importantly, however, while the influence of the adrenal response on local inflammation induced by *E. faecalis* infection is clear, the overall contribution of adrenal regulation to a potentially lethal outcome of infection is significantly less clear. In this regard, only one of the two *E. faecalis* strains examined manifested significant changes in LD₅₀s depending upon the functional status of the adrenal gland or the administration of exogenous glucocorticoids to regulate inflammation. The implications of these observations for the treatment of patients with *E. faecalis* infections may be significant.

In a previous publication, we demonstrated that *E. faecalis* was substantially less virulent than a wide variety of other bacteria tested, as indicated by markedly higher LD₅₀s in an experimental mouse model of infection (25). Additionally, we reported that i.p. infection with *E. faecalis* elicited a muted localized TNF- α response and failed to elicit a detectable systemic TNF- α response, even in mice that died following infection. Paradoxically, however, *E. faecalis* was capable of inducing a rapid and robust TNF- α response from cultured macrophages in vitro, indicating that the weak host TNF- α response to *E. faecalis* infection was not attributable to the inability of the organism to activate macrophages. We also demonstrated that *E. faecalis* infection induced rapid and robust localized and systemic IL-6 responses. One regulatory function attributed to IL-6 is the ability to suppress an ongoing TNF- α response (1, 13). In the typical response to bacterial infection, TNF- α production temporally precedes IL-6 production (11, 24, 33, 35). The early release of TNF- α stimulates IL-6 production, which functions as part of a negative regulatory feedback pathway to turn off TNF- α production (9, 18). IL-6 can suppress TNF- α production directly, by acting on TNF- α -producing cells, or indirectly, by stimulating corticosterone production (1, 4, 32, 36). Our data indicated that the in vivo response of mice to *E. faecalis* differs from the typical host response to bacterial infection, as circulating IL-6 levels increase rapidly, despite undetectable

serum TNF- α . Under these conditions, it is possible that early, sustained IL-6 production might induce a glucocorticoid response that would suppress TNF- α production before a significant TNF- α response can be generated. The results of the current study, summarized above, support the conclusion that the endogenous glucocorticoid response to *E. faecalis* infection suppresses localized TNF- α production within the peritoneal cavity. Neither adrenalectomized nor sham-adrenalectomized mice infected i.p. with *E. faecalis* generated a systemic TNF- α response, however, implicating factors other than endogenous glucocorticoid production in the failure of *E. faecalis* to induce such a response.

It is well documented that bacterial infections induce a host glucocorticoid response that suppresses TNF- α production, so the finding that this also occurs within the peritoneal cavities of *E. faecalis*-infected mice is not particularly surprising (4, 15, 29, 32, 34, 36, 37). The unusual host TNF- α response to *E. faecalis* infection, however, may provide further insight into the relationship between host adrenal response and TNF- α production. TNF- α remained undetectable in the sera of both adrenalectomized and sham-adrenalectomized *E. faecalis*-infected mice and therefore could not have been delivered to the peritoneum from the systemic circulation. It is therefore reasonable to conclude that any TNF- α detected within the peritoneum of *E. faecalis*-infected mice was produced locally. Consequently, local factors would also have to account for the depressed peritoneal TNF- α levels in sham-adrenalectomized compared to adrenalectomized mice. A literature search did not provide insights into this concept, however, as we were unable to identify any publications in which glucocorticoids were detected within the peritoneal cavity. We therefore opted to directly measure corticosterone levels in peritoneal-lavage fluid and demonstrated here that peritoneal corticosterone was undetectable in vehicle-treated controls but began to increase within an hour, peaked at 3 h, and returned to normal by 7 h after *E. faecalis* infection. It is likely that localized inflammation within peritoneal blood vessels permitted passage of corticosterone from the blood into the peritoneum, as serum corticosterone also began increasing within an hour, peaked at 3 h, and returned to near normal by 7 h after *E. faecalis* infection. This corticosterone then suppressed TNF- α production by cells, presumably macrophages, within the peritoneum.

The potential clinical significance of the adrenal-glucocorticoid response to *E. faecalis* was demonstrated in lethality studies. Compared with sham-adrenalectomized controls, adrenalectomized mice were markedly more susceptible to potentially lethal *E. faecalis* CP-1 infection, as demonstrated by a >100-fold decrease in the LD₅₀. Further, the increased susceptibility of adrenalectomized mice to *E. faecalis* CP-1 infection was partially reversed by pretreatment with the exogenous glucocorticoid dexamethasone. These data support the concept that *E. faecalis* CP-1 induces an endogenous glucocorticoid response that protects against potentially lethal infection. Surprisingly, similar results were not attained with *E. faecalis* K9. Adrenalectomy only minimally increased susceptibility to potentially lethal *E. faecalis* K9 infection (an ~3-fold decrease in the LD₅₀).

Potential pathophysiologic explanations for the differential impacts of host adrenal responses on susceptibility to *E. faecalis* CP-1 versus K9 infections are not immediately apparent. Al-

though the corticosterone response to *E. faecalis* CP-1 is significantly greater than the response to *E. faecalis* K9, the extents to which this response inhibits local TNF- α production within the peritoneal cavity are similar for the two organisms. It is clear, however, that the host adrenal-glucocorticoid response to *E. faecalis* CP-1 is protective, while the response to *E. faecalis* K9 is not protective. To further explore this phenomenon, we inoculated mice i.p. with sublethal doses of *E. faecalis* CP-1, to induce the potential protective effects, 1 hour prior to inoculation with graded doses of *E. faecalis* K9. Our goal in this series of experiments was to determine whether the response to *E. faecalis* CP-1, which protects against homologous infection, could also elicit that protective effect in mice infected with potentially lethal doses of the heterologous *E. faecalis* K-9 strain. The LD₅₀s for mice infected with *E. faecalis* K9 were not altered by prior *E. faecalis* CP-1 infection. These results support the conclusion that *E. faecalis* K9 is more resistant to potentially protective adrenal-glucocorticoid host responses than *E. faecalis* CP-1. This may partially explain our previous observations, confirmed here, that *E. faecalis* K9 is more virulent than *E. faecalis* CP-1, as determined by substantially lower LD₅₀s (25).

One possible explanation for these observations would derive from further consideration of the data in Fig. 4. We noted earlier that the marked increase in the sensitivity to *E. faecalis* CP-1 infection brought about by adrenalectomy was only partially reversed by dexamethasone pretreatment. It may not be simply coincidental that the LD₅₀ for adrenalectomized, dexamethasone-treated *E. faecalis* CP-1-infected mice is virtually identical to the LD₅₀ for *E. faecalis* K9, which is essentially refractory to the presence or absence of glucocorticoids. It is possible, therefore, that the cumulative virulence of various *E. faecalis* strains can be attributed to a component that is relatively refractory to the intrinsic glucocorticoid response (a property shared by both *E. faecalis* CP-1 and K9), as well as a glucocorticoid-sensitive component (a property unique to *E. faecalis* CP-1). Based on this concept, the determination of effective therapeutic intervention strategies would depend critically upon an understanding of the virulence properties of the infecting *E. faecalis* strain.

Recent attention has been directed toward the relatively high rate of adrenal insufficiency in critically ill patients (7, 20, 27). The overall incidence of adrenal insufficiency in such patients approximates 30%, though this varies significantly with the patient population studied and the criteria used to define adrenal insufficiency (20). Enterococcal sepsis is most likely to occur in patients with critical underlying illness; the most important independent predictors of mortality in patients with enterococcal bacteremia were two indices of poor overall health status (APACHE II and the Weighted Comorbidity Index) (31). Consequently, it is anticipated that a relatively high proportion of patients at increased risk for enterococcal sepsis will exhibit adrenal insufficiency. We have demonstrated in an experimental animal model that mortality rates are substantially increased in adrenalectomized animals infected with one of two *E. faecalis* strains. Although poor overall health is an independent predictor of mortality in patients with enterococcal bacteremia, the pathogenic mechanisms that account for this increased mortality have not been defined. If, as in our experimental model, adrenal insufficiency contributes to the

pathogenesis of some enterococcal infections in humans, exogenous glucocorticoid therapy could have a potentially significant beneficial effect.

In summary, we have demonstrated that an endogenous glucocorticoid response to *E. faecalis* infection is likely to be responsible for the relatively weak, localized TNF- α response elicited by the organism in an experimental mouse model. Further, this glucocorticoid response also protected mice from potentially lethal *E. faecalis* infections with one of two strains tested. These findings are of considerable potential importance for furthering our understanding of the pathogenesis of this opportunistic pathogen. Adrenal insufficiency in critically ill patients could predispose them to potentially lethal septic responses to opportunistic *E. faecalis* strains that would have little potential to induce such responses in patients with normal adrenal function.

ACKNOWLEDGMENTS

This work was supported by Public Health Service Grants AI46493 (C.J.P.), AI54962 and GM50870 (N.Q.), and AI44936 (D.C.M.) from the National Institutes of Health.

We are grateful to Eleanor Zuvanich for her expert technical assistance and to Richard Silverstein for his helpful discussions.

REFERENCES

- Aderka, D., J. Le, and J. Vilcek. 1989. IL-6 inhibits lipopolysaccharide-induced tumor necrosis factor production in cultured human monocytes, U937 cells, and in mice. *J. Immunol.* **143**:3517–3523.
- Balk, R. A. 2003. Steroids for septic shock: back from the dead? *Chest* **123**:490S–499S.
- Bertini, R., M. Bianchi, and P. Ghezzi. 1988. Adrenalectomy sensitizes mice to the lethal effects of interleukin 1 and tumor necrosis factor. *J. Exp. Med.* **167**:1708–1712.
- Bethin, K. E., S. K. Vogt, and L. J. Muglia. 2000. Interleukin-6 is an essential, corticotropin-releasing hormone-independent stimulator of the adrenal axis during immune system activation. *Proc. Natl. Acad. Sci. USA* **97**:9317–9322.
- Bone, R. C., C. J. Grodzin, and R. A. Balk. Sepsis: a new hypothesis for pathogenesis of the disease process. *Chest* **112**:235–243.
- Butler, L. D., N. K. Layman, P. E. Riedl, J. Shellhaas, G. F. Evans, and S. H. Zuckerman. 1989. Neuroendocrine regulation of in vivo cytokine production and effects: I. In vivo regulatory networks involving the neuroendocrine system, interleukin-1 and tumor necrosis factor- α . *J. Neuroimmunol.* **24**:143–153.
- Cooper, M. S., and P. M. Stewart. 2003. Current concepts: corticosteroid insufficiency in acutely ill patients. *N. Engl. J. Med.* **348**:727–734.
- Deans, K. J., M. Haley, C. Natanson, P. Q. Eichacker, and P. C. Minneci. 2005. Novel therapies for sepsis: a review. *J. Trauma* **58**:867–874.
- Di Santo, E., T. Alonzi, V. Poli, E. Fattori, C. Toniatti, M. Sironi, P. Ricciardi-Castagnoli, and P. Ghezzi. 1997. Differential effects of IL-6 on systemic and central production of TNF: a study with IL-6-deficient mice. *Cytokine* **9**:300–306.
- Edmond, M. B., J. F. Ober, J. D. Dawson, D. L. Weinbaum, and R. P. Wenzel. 1996. Vancomycin-resistant enterococcal bacteremia: natural history and attributable mortality. *Clin. Infect. Dis.* **23**:1234–1239.
- Fong, Y., K. J. Tracey, L. L. Moldawer, D. G. Hesse, K. B. Manogue, J. S. Kenney, A. T. Lee, G. C. Kuo, A. C. Allison, S. F. Lowry, and A. Cerami. 1989. Antibodies to cachectin/tumor necrosis factor reduce interleukin 1 β and interleukin 6 appearance during lethal bacteremia. *J. Exp. Med.* **170**:1627–1633.
- Gonzalez, J. C., D. C. Johnson, D. C. Morrison, M. A. Freudenberg, C. Galanos, and R. Silverstein. 1993. Endogenous and exogenous glucocorticoids have different roles in modulating endotoxin lethality in D-galactosamine-sensitized mice. *Infect. Immun.* **61**:970–974.
- Hack, C. E., L. A. Aarden, and L. G. Thijs. 1997. Role of cytokines in sepsis. *Adv. Immunol.* **66**:101–195.
- Jett, B. D., M. K. Huycke, and M. S. Gilmore. 1994. Virulence of enterococci. *Clin. Microbiol. Rev.* **7**:462–478.
- Joyce, D. A., G. Gimblett, and J. H. Steer. 2001. Targets of glucocorticoid action on TNF- α release by macrophages. *Inflamm. Res.* **50**:333–340.
- Kapcala, L. P., T. Chautard, and R. L. Eskay. 1995. The protective role of the hypothalamic-pituitary-adrenal axis against lethality produced by immune, infectious, and inflammatory stress. *Ann. N. Y. Acad. Sci.* **771**:419–433.

17. Koo, D. J., D. Jackman, I. Chaudry, and P. Wang. 2001. Adrenal insufficiency during the late stage of polymicrobial sepsis. *Crit. Care Med.* **29**:618–622.
18. Kozak, W., M. J. Kluger, D. Soszynski, C. A. Conn, K. Rudolph, L. R. Leon, and H. Zheng. 1998. IL-6 and IL-1 β in fever. Studies using cytokine-deficient (knockout) mice. *Ann. N. Y. Acad. Sci.* **856**:33–47.
19. Landry, S. L., D. L. Kaiser, and R. P. Wenzel. 1989. Hospital stay and mortality attributed to nosocomial enterococcal bacteremia: a controlled study. *Am. J. Infect. Control.* **17**:323–329.
20. Marik, P. E., and G. P. Zaloga. 2002. Adrenal insufficiency in the critically ill: a new look at an old problem. *Chest* **122**:1784–1796.
21. Meduri, G. U., and S. Kanangat. 1998. Glucocorticoid treatment of sepsis and acute respiratory distress syndrome: time for a critical reappraisal. *Crit. Care Med.* **26**:630–633.
22. Micek, S. T., R. A. Shah, and M. H. Kollef. 2003. Management of severe sepsis: integration of multiple pharmacologic interventions. *Pharmacotherapy* **23**:1486–1496.
23. Mundy, L. M., D. F. Sahm, and M. Gilmore. 2000. Relationships between enterococcal virulence and antimicrobial resistance. *Clin. Microbiol. Rev.* **13**:513–522.
24. Nakane, A., M. Okamoto, M. Asano, M. Khoanawa, and T. Minagawa. 1995. Endogenous gamma interferon, tumor necrosis factor, and interleukin-6 in *Staphylococcus aureus* infection in mice. *Infect. Immun.* **63**:1165–1172.
25. Papsian, C. J., R. Silverstein, J. J. Gao, D. M. Bamberger, and D. C. Morrison. 2002. Anomalous role of TNF- α in experimental enterococcal infection. *Infect. Immun.* **70**:6628–6637.
26. Reed, L. J., and H. A. Muench. 1938. A simple method of estimating 50 percent endpoints. *Am. J. Hyg.* **27**:493–497.
27. Rivers, E. P., M. Gaspari, G. A. Saad, M. Mlynarek, J. Fath, H. M. Horst, and J. Wortsman. 2001. Adrenal insufficiency in high-risk surgical ICU patients. *Chest* **119**:889–896.
28. Sands, K. E., D. W. Bates, P. N. Lanken, P. S. Graman, P. L. Hibberd, K. L. Kahn, J. Parsonnet, R. Panzer, E. J. Orav, and D. R. Snyderman. 1997. Epidemiology of sepsis syndrome in eight academic medical centers. *JAMA* **238**:234–240.
29. Silverstein, R., and D. C. Johnson. 2003. Endogenous versus exogenous glucocorticoid responses to experimental bacterial sepsis. *J. Leukoc. Biol.* **73**:417–427.
30. Soni, A., G. M. Pepper, P. M. Wyrwinski, N. E. Ramirez, R. Simon, T. Pina, H. Gruenspan, and C. E. Vaca. 1995. Adrenal insufficiency occurring during septic shock: incidence, outcome and relationship to peripheral cytokine levels. *Am. J. Med.* **98**:266–271.
31. Stroud, L., J. Edwards, L. Danzig, D. Culver, and R. Gaynes. 1996. Risk factors for mortality associated with enterococcal bloodstream infections. *Infect. Control Hosp. Epidemiol.* **17**:576–580.
32. Turnbull, A. V., and C. L. Rivier. 1999. Regulation of the hypothalamic-pituitary-adrenal axis by cytokines: actions and mechanisms of action. *Physiol. Rev.* **79**:1–71.
33. van der Poll, T., M. Levi, S. J. H. van Deventer, H. ten Cate, B. L. Haagmans, B. J. Biemond, H. R. Buller, C. E. Hack, and J. W. ten Cate. 1994. Differential effects of anti-tumor necrosis factor monoclonal antibodies on systemic inflammatory responses in experimental endotoxemia in chimpanzees. *Blood* **83**:446–451.
34. van der Poll, T., and S. J. H. van Deventer. 1998. The role of interleukin 6 in endotoxin-induced inflammatory responses. *Prog. Clin. Biol. Res.* **397**:365–377.
35. van der Poll, T., and S. J. H. van Deventer. 1999. Cytokines and anticytokines in the pathogenesis of sepsis. *Infect. Dis. Clin. N. Am.* **13**:413–426.
36. van Enkevort, F. H. J., D. G. J. Sweep, P. N. Span, M. G. Netea, A. R. M. M. Hermus, and B. J. Kullberg. 2001. Reduced adrenal response and increased mortality after systemic *Klebsiella pneumoniae* infection in interleukin-6-deficient mice. *Eur. Cytokine Netw.* **12**:581–586.
37. Vernihaki, M., P. Dikkes, A. Carrigan, and K. P. Karalis. 2001. Corticotropin-releasing hormone regulates IL-6 expression during inflammation. *J. Clin. Invest.* **108**:1159–1166.
38. Zaloga, G. P. 2001. Sepsis-induced adrenal deficiency syndrome. *Crit. Care Med.* **29**:688–690.