

Lethal and Edema Toxins in the Pathogenesis of *Bacillus anthracis* Septic Shock Implications for Therapy

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Recent research regarding the structure and function of *Bacillus anthracis* lethal (LeTx) and edema (ETx) toxins provides growing insights into the pathophysiology and treatment of shock with this lethal bacteria. These are both binary-type toxins composed of protective antigen necessary for their cellular uptake and either lethal or edema factors, the toxigenic moieties. The primary cellular receptors for protective antigen have been identified and constructed and key steps in the extracellular processing and internalization of the toxins clarified. Consistent with the lethal factor's primary action as an intracellular endopeptidase targeting mitogen-activated protein kinase kinases, growing evidence indicates that shock with this toxin does not result from an excessive inflammatory response. In fact, the potent immunosuppressive effects of LeTx may actually contribute to the establishment and persistence of infection. Instead, shock with LeTx may be related to the direct injurious effects of lethal factor on endothelial cell function. Despite the importance of LeTx, very recent studies show that edema factor, a potent adenyl cyclase, has the ability to make a substantial contribution to shock caused by *B. anthracis* and works additively with LeTx. Furthermore, ETx may contribute to the immunosuppressive effects of LeTx. Therapies under development that target several different steps in the cellular uptake and function of these two toxins have been effective in *in vitro* and *in vivo* systems. Understanding how best to apply these agents clinically and how they interact with conventional treatments should be goals for future research.

Keywords: anthrax; toxin; shock; treatment

Inhalational *Bacillus anthracis* is a serious bioterrorism-related health threat today (1). In the 2001 outbreak in the United States, the uniform fatality in patients with shock despite aggressive hemodynamic support as well as the pronounced hemoconcentration and recurrent pleural effusions noted support observations that the pathophysiology of shock with this infection may differ from more common types of sepsis (1, 2). Whether supportive measures conventionally applied in septic shock are as effective for *B. anthracis* is unclear. It is apparent, however, that lethal toxin (LeTx), composed of lethal factor (LF) and protective antigen (PA), and edema toxin (ETx), made up of edema factor (EF) and PA, are important for this microbe's pathogenesis (3, 4). Past and recent work directed at the structure

and function of these two toxins provides increasing insights into both the mechanisms and treatment of *B. anthracis* shock (5). Some of the work presented in this article has been presented previously in abstract form (6).

THE BINARY NATURE OF *B. ANTHRACIS* LETHAL AND EDEMA TOXINS

Smith and Keppie first showed that plasma from *B. anthracis*-infected animals produced lethality in normal animals (7). Crude preparations of toxin prepared from such plasma were associated with extravascular edema, hypothermia, and hemoconcentration similar to that in infected animals. Antiserum developed against these preparations prevented lethality. In experiments by others, the serum of rhesus monkeys infected with anthrax was progressively more toxic to rats as bacterial count in donor animals rose, and was also lethal when given to uninfected monkeys (8).

In the first experiments describing the three *B. anthracis* toxin components, EF, initially designated factor I, produced local skin edema and, in larger doses, lethality, but only when given with a second factor (factor II), now known as PA (9). LF, first designated factor III, was highly lethal when combined with PA but did not produce skin edema. The combination of all three factors was most lethal and produced many of the characteristics of unfractionated toxin and bacterial infection. Vaccination with the combination of components was protective during bacterial infection (10). Later studies in mice with mutant *B. anthracis* strains supported the relative roles of these toxins during infection (3). Mutant strains lacking either PA or LF were not lethal. Strains lacking EF still produced lethality but this was not as great as with the parental strain producing all three components.

On the basis of these and other studies, lethal and edema toxins are now recognized to be binary or A-B-type toxins (11). PA is the "B" component that initiates cell binding and uptake of each toxin (Figure 1). LF and EF are the "A," or toxigenic, components, which attach to PA on the cell surface and are then delivered into the cytosol where they exert their effects. These three proteins are encoded on the pXO1 plasmid, which is required for bacterial virulence.

PA AND ITS HOST CELLULAR RECEPTORS

PA is an 83-kD (PA₈₃) four-domain protein (12). Circulating PA binds via domain IV to one of at least two different cellular receptors, including tumor endothelial marker 8 (TEM8) and capillary morphogenesis gene 2 (CMG2) (13, 14) (Figure 1). The PA precursor molecule then undergoes furin protease-mediated cleavage in domain I into 20 kD (PA₂₀) and 63 kD (PA₆₃) proteins (15). After PA₂₀ release, remaining PA₆₃ units form a heptamer, also termed a prepore, that localizes to lipid raft regions in host cell membranes (16). Between one and three molecules of

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circulating LF or EF bind competitively to high-affinity sites on individual heptamers (17). Oligomerization is necessary for LF/EF binding. This combination then undergoes endocytosis and is progressively acidified. At a pH of 5.5, the prepore forms a barrel-like pore in the endosomal membrane through which LF and EF are translocated into the cytosol (18).

Both TEM8 and CMG2, the two host cellular receptors to which PA binds, have been associated with a variety of normal tissues, including heart, lung, small intestine, spleen, liver, kidney, skeletal muscle, and skin (13, 14, 19). Common to both proteins and with a high degree of homology are von Willebrand factor A or integrin inserted (VWA/I) extracellular domains (20). These domains contain metal ion-dependent adhesion sites (MIDAS) normally involved in binding to adhesion molecules or extracellular matrix proteins. Domain IV of PA competes with natural ligands for these MIDAS binding sites.

LF AND TOXIN

Structure and Function

LF is a 90-kD Zn^{2+} protease with four folding domains (21). Domain I binds to PA prior to cell uptake (Figure 1). The only presently recognized substrates for LF in the cytosol are the mitogen-activated protein kinase kinases (MAPKKs or MEKs) 1 to 4, 6, and 7, which are bound by domains II and III of LF and cleaved at the N-terminal ends by domain IV (5, 22). This cleavage prevents activation of each of the MAPKKs and has the potential to disrupt downstream signaling, which is essential for a variety of normal cell functions, including activation of immune and stress responses.

Cardiovascular Effects of LeTx

Despite LeTx's association with the pathogenesis of *B. anthracis*, few studies have investigated its cardiovascular effects. In early studies in guinea pigs, LeTx was reported to produce extravasation of fluid and a shocklike state, but hemodynamics were not measured (23). Bolus administration of toxin in rats caused pulmonary edema, pleural fluid collections, hemoconcentration, hypoxemia, and rapid lethality (24–26). In one such study, heart rate and blood pressure decreased immediately before death, reportedly secondary to respiratory distress (26). Intravenous administration of LeTx in rhesus monkeys reduced blood pressure and heart rate immediately before death, reportedly second-

ary to central respiratory depression (27). After these early investigations, no additional studies describing this toxin's hemodynamic effects were reported over the ensuing 30 years. However, aspects of the disease noted during the 2001 outbreak, while consistent with some of these prior observations, such as the hemoconcentration and extravasation of fluid, were not consistent with others. For example, shock in patients occurred well before death in nonsurvivors and was not initially a result of respiratory failure.

After the 2001 outbreak, our laboratory developed a rat model to further investigate LeTx-induced shock (28). Rather than administering toxin as a bolus, however, it was infused over 24 hours to better simulate the pattern of release during bacterial infection. Using LeTx doses designed to produce 50% lethality rates, death occurred in some rats starting at 10 to 12 hours and rats continued to die over the next 24 to 48 hours. Compared with control rats, LeTx produced reductions in blood pressure and heart rate several hours before initial lethality, which worsened in nonsurvivors but recovered in survivors (Figures 2A and 2B). Although toxin was associated with evidence of hemoconcentration, pleural fluid collections, and tissue hypoperfusion, respiratory failure and arterial hypoxemia were not primary contributors to death (28) (Figure 2C). Histologic studies did not demonstrate evidence of pulmonary or myocardial injury. In subsequent studies, inhibition of PA with monoclonal antibody administered up to 6 hours after the initiation of LeTx significantly increased blood pressure and heart rate, reduced evidence of hemoconcentration, and improved survival (29) (Figure 3). Thus, in contrast to earlier studies, findings in this rat model suggest that LeTx can produce shock independent of pulmonary dysfunction.

Potential Mediators and Mechanisms of LeTx-induced Shock

The mediators and mechanisms underlying shock with LeTx remain unclear. Earlier studies suggested that consistent with the excessive inflammatory response some bacterial toxins elicit, LeTx stimulated macrophage production of IL-1 and tumor necrosis factor (TNF) that ultimately led to cell lysis and cytokine release (30). Mice depleted of macrophages with silica were resistant to the lethal effects of LeTx, whereas supplementation with cultured macrophages restored these animals' sensitivity. Finally, inhibition of IL-1, and to a lesser extent TNF, improved survival with LeTx. Subsequent studies, however, have indicated that neither excessive cytokine nor nitric oxide (NO) production

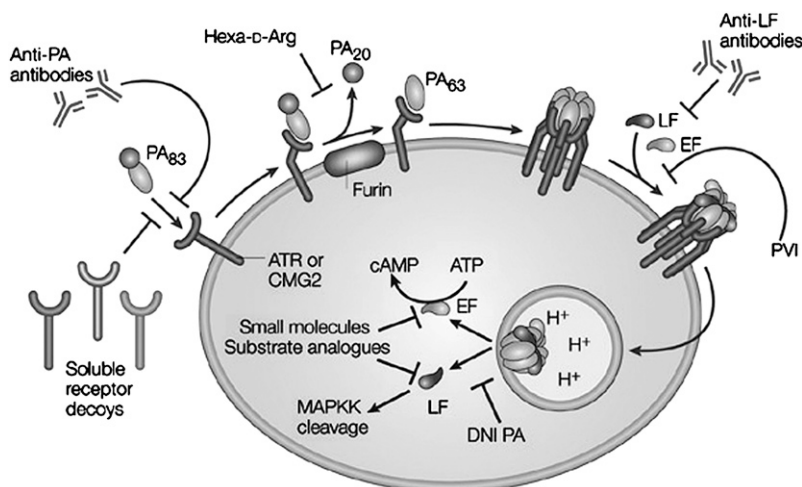


Figure 1. Summary of the fundamental steps in lethal and edema toxin uptake by host cells, the intracellular effects of the toxins, and the points in the process that have provided targets for antitoxin therapies. ATR = anthrax toxin receptor (tumor endothelial marker-8); CMG2 = capillary morphogenesis gene 2; DNI = dominant-negative inhibitor; EF = edema factor; Hexa-D-Arg = hexa-D-arginine; LF = lethal factor; MAPKK = mitogen-activated protein kinase; PA = protective antigen; PVI = polyvalent inhibitor. Adapted by permission from Reference 5.

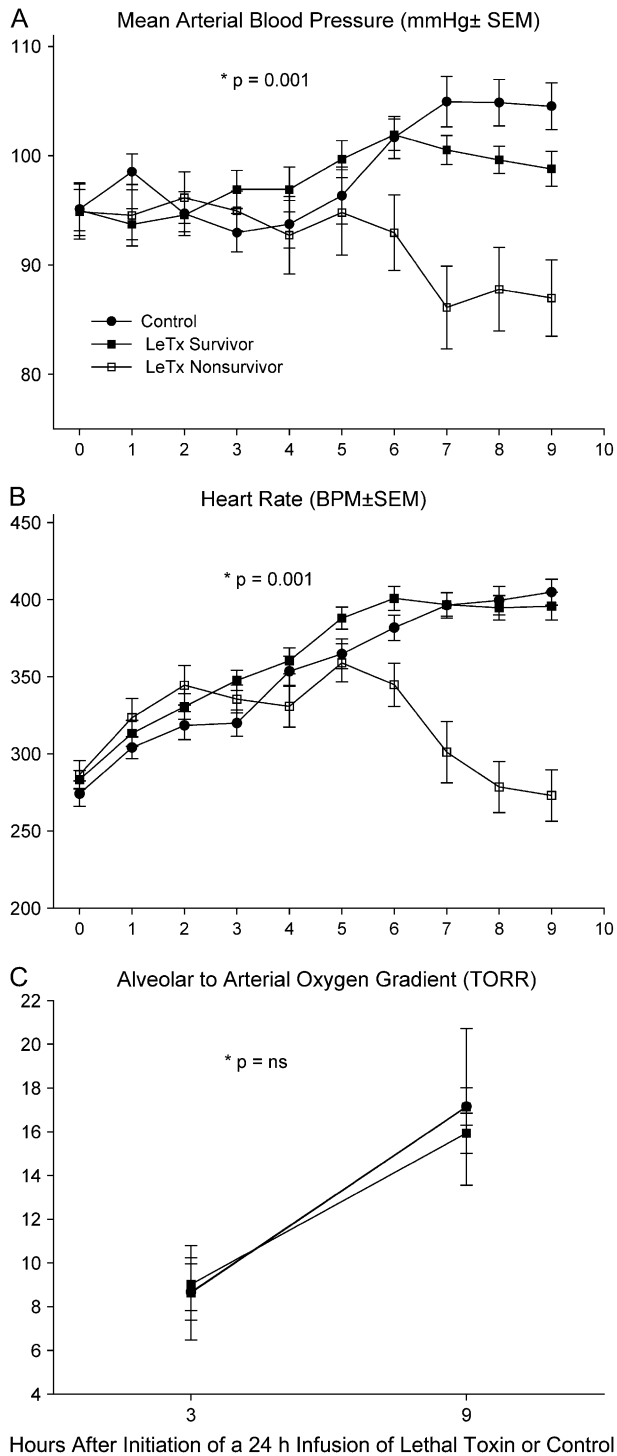


Figure 2. Serial (A) mean arterial blood pressures, (B) heart rates, and (C) alveolar arterial oxygen gradients in surviving and nonsurviving rats after initiation of a 24-h lethal toxin (LeTx) infusion or in animals receiving diluent only (controls). Reductions in blood pressure and heart rate were first evident in nonsurvivors 4 to 5 h before initial lethality at 10 h. Arterial hypoxemia was not evident. *p values are for the time and group interaction.

contributes to the pathogenesis of LeTx. In fact, consistent with its inhibitory effects on MAPKK function, LeTx may suppress the inflammatory response. In *in vitro* studies in murine macrophages, LeTx did not stimulate TNF- α or IL-1 β production

by macrophages, and inhibited cytokines, IL-1 β mRNA, and NO production after LPS challenge (31, 32). In murine and human macrophages, MAPKK cleavage by LeTx inhibited LPS-stimulated IFN-regulatory factor 3 (IRF3) (33). In T cells, LeTx blocked IL-2 production through MAPKK inhibition (34). LeTx-treated murine dendritic cells that were stimulated with LPS did not up-regulate costimulatory molecules, secreted greatly diminished inflammatory cytokines, and did not stimulate T cells *in vivo* (35). In *in vivo* studies in BALB/c and C57BL/6 mice, lethal LeTx doses produced small increases in IL-1 β in the former but none in the latter strain, whereas TNF levels were not altered in either strain (36). In our rat model, compared with 24-hour infusions of LPS, similarly lethal doses of LeTx were associated with significantly reduced inflammatory cytokine and NO production (Figure 4) (28). In further studies, sublethal LeTx doses inhibited LPS and *Escherichia coli*-stimulated cytokine (Figure 5A) and NO production (37).

Growing evidence does suggest that direct effects of LeTx on endothelial cell function could contribute to shock. Cultured human endothelial cells exposed to LeTx underwent apoptosis possibly via extracellular signal-regulated protein kinase (ERK) pathway inhibition (38). Also, LeTx decreased the barrier function of human lung microvascular endothelial cells as assessed with transendothelial electrical resistance and labeled albumin measures (39). This was accompanied by altered distribution of actin fibers and vascular endothelial cadherin necessary for normal barrier function. Finally, intradermal LeTx administration resulted in vascular leak within 15 to 25 minutes in a mouse model using the Miles Evans blue assay (40). Consistent with this evidence, LeTx challenge in animal models is associated with extravascular fluid collections and hemoconcentration similar to that observed during live bacterial infection (28, 36, 40, 41).

LeTx may also contribute to shock via its effects on glucocorticoid receptor (GR) function. In *in vitro* studies, LeTx inhibited transactivation of dexamethasone-induced GR in both a transfection system and in cells endogenously expressing GR (42). In *in vivo* studies, LeTx inhibited dexamethasone-induced liver tyrosine aminotransferase activity in BALB/c mice. Despite these suppressive effects, however, dexamethasone treatment worsened outcome in LeTx-challenged BALB/c mice.

Immunosuppressive Effects of LeTx

Although LeTx may not produce shock via stimulation of inflammatory host mediators, its inhibition of these same mediators as well as other components in host defense may contribute to the establishment and persistence of *B. anthracis* infection. In higher doses, LeTx causes macrophage lysis, although this appears species and strain specific and is not required to produce lethality or shock (28, 36). The sensitivity of murine macrophages to lysis by LeTx is determined by the gene Nalp1b on chromosome 11 (43). Nalp1b activates the caspase-1 response to LeTx, which in turn leads to macrophage death. In sublytic doses, LeTx can also cause apoptosis of activated macrophages via MAPKK or caspase pathways (44, 45).

In addition to its inhibitory effects on cytokines associated with the innate immune response outlined above, LeTx impairs neutrophil function. Neutrophils exposed to LeTx generated significantly less superoxide anion (O₂⁻) in response to LPS compared with LeTx-free cells (46). LeTx was also shown to interfere with actin assembly and to impair neutrophil chemotaxis (47). This is consistent with the finding that LeTx challenge causes intravascular aggregation of neutrophils in mice (36).

Studies in our rat model support the potent inhibitory effect LeTx may have on innate immune responses and protective host defense mechanisms during infection (37). Reductions in inflammatory cytokines and NO release with sublethal LeTx

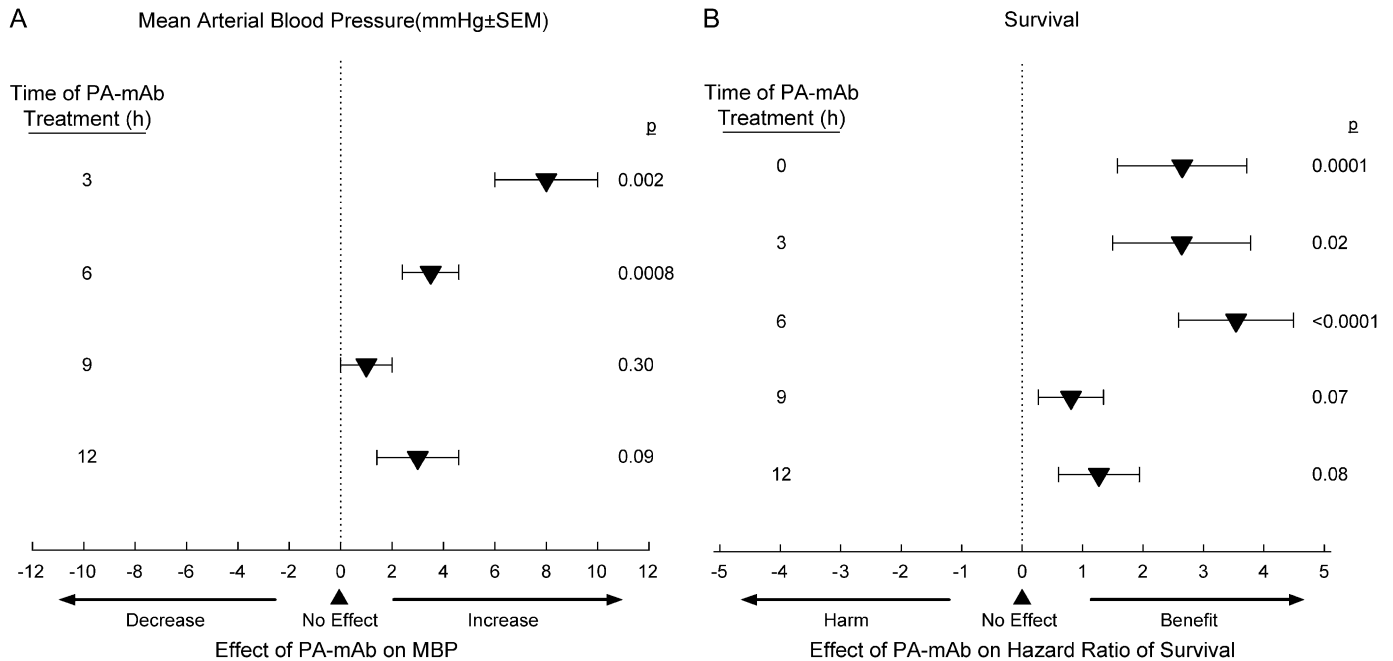


Figure 3. The effects of treatment with protective antigen-directed monoclonal antibody (PA-mAb) compared with placebo started at the time of or up to 12 h after the start of a 24-h LeTx infusion on (A) mean arterial blood pressure (MBP) and (B) the hazard ratio of survival in rats. Blood pressures were measured and averaged over the 12 h after treatment, and survival was monitored for 168 h. PA-mAb increased blood pressure and survival either significantly or in trends approaching it even if delayed for up to 12 h after the start of LeTx (29).

doses in animals challenged with LPS or intratracheal *E. coli* were associated with increased blood pressures, although these were greater with the former than the latter (Figures 5A and 5B). However, whereas sublethal LeTx doses improved survival with LPS, a noninfectious challenge, it decreased survival with *E. coli* in patterns that were significantly different (Figure 5C). Worsened outcome with *E. coli* infection was also associated with evidence of reduced alveolar leukocyte recruitment that would normally be critical for extravascular microbial clearance (37).

LeTx alters adaptive immunity as well. In T-cell lymphocytes, LeTx disrupted antigen receptor signaling through the CD3 and CD28 receptors (48, 49). Furthermore, in human CD4⁺ T cells, LeTx inhibited IL-2 production and IL-2-dependent T-cell proliferation after T-cell receptor (TCR) stimulation (49). In other studies, MAPKK cleavage by LeTx inhibited B-cell proliferation and IgM production (50).

EF AND TOXIN

Structure and Function

EF, like LF, is transported into the cell via the PA heptamer (Figure 1) (51). The binding site on EF for PA is a seven-amino-acid (residues 136–142) chain identical to residues 147–153 on LF. EF mutations in Tyr 137, Tyr 138, Ile 140, and Lys 142 block interaction with PA (52). EF is an adenylyl cyclase that leads to increased cAMP in host cells (53). It is distinct from mammalian adenylyl cyclase but shares a common two-metal-ion catalytic mechanism (54). EF's catalytic activity requires the binding of calmodulin, a eukaryotic calcium binding protein (54). This leads to a change of calmodulin activity: two C-terminal Ca²⁺ binding sites show increased affinity and loss of cooperativity, whereas the N-terminal domain has a reduction in affinity and an increase in cooperativity (55). The EF-calmodulin complex catalyzes the synthesis of cAMP in host cells (56). The production of cAMP

is dependent on an influx of calcium, and cAMP accumulation is prevented by calcium channel antagonists or the absence of calcium. EF has also now been shown to stimulate PA receptor function (57).

Cardiovascular Effects of ETx

After early findings that ETx was less lethal than LeTx, there were no further studies assessing its *in vivo* effects until two very recent ones (10, 58, 59). In one, administration of ETx in BALB/cJ mice produced pathologic lesions, including lymph node and focal gastrointestinal tract hemorrhage, adrenal damage, and intestinal fluid accumulation, similar to those reported in human series (58). Pleural effusions were absent, however. Compared with prior studies with LeTx in the same model, comparable doses of ETx produced greater lethality. Hemodynamic measures close to the time of death with a 100% lethal dose of ETx showed hypotension and, despite this toxin's potent adenylyl cyclase activity, bradycardia.

Using the rat model developed in our lab, the effects of recombinant preparations of ETx and LeTx both alone and together as 24-hour infusions were compared (59). The range of doses of ETx alone that produced increasing lethality (0–100%) was tenfold greater than that of LeTx. However, similarly lethal doses of ETx produced greater and earlier hypotension than LeTx and increased rather than decreased heart rates (Figures 6A and 6B). Lethal doses of ETx were not associated with arterial hypoxemia. Furthermore, ETx and LeTx, when combined in doses that were either similar in weight or lethality, had effects that were additive (Figure 7). These and other findings therefore suggest that ETx may be as important as LeTx in the development of shock during *B. anthracis* infection and should be viewed as a possible therapeutic target.

Potential Mediators and Mechanisms of ETx-induced Shock

In light of its effects on tissue edema after local administration, extravasation of fluid is one possible mechanism for the shock occurring with ETx. However, in a mouse model, although highly lethal ETx doses produced intestinal intraluminal fluid accumulation and hemoconcentration, histologic analysis of several organs did not show significant extravasation of fluid (58). In our rat model, despite studying a range of ETx doses, hemoconcentration was not evident (59). Thus, to what extent shock with ETx is related to extravasation of fluid is unclear. What is evident, however, is that ETx has potent adenylyl cyclase activity (53–56). This characteristic provides a strong basis for the cardiovascular dysfunction it has been shown to produce in recent *in vivo* models (60). Increases in cellular cAMP and activation of cAMP-dependent protein kinase could result both in arterial relaxation and shock as well as the tachycardia noted in our model.

ETx, similar to LeTx and consistent with its effects on intracellular cAMP levels, has also not been associated with the excessive inflammatory cytokine or NO production typically associated with other types of sepsis (58, 59). In fact, ETx may have immunosuppressive effects similar to LeTx. In *in vitro* or *in vivo* studies, ETx appeared to inhibit TNF- α , IFN- γ , IL-12p70, monocyte chemoattractant protein (MCP)-1, macrophage inflammatory protein (MIP)-1 α , and MIP-1 β (49, 58, 61–63). However, ETx has been associated with increases in IL-6, granulocyte colony-stimulating factor, exotoxin, keratinocyte-derived chemokine (KC), IL-10, IL-1 and monocyte-selective chemokine (JE)/MCP-1 (49, 58, 62, 63). This toxin has also been shown to inhibit activation and proliferation of CD4⁺ T cells (49). Neutrophils exposed to ETx were unable to phagocytize Sterne strain *B. anthracis* (64). In our rat model, ETx was not associated with any consistent increase in cytokine or NO release (59). However, circulating neutrophil numbers were increased and lymphocytes decreased over the 24 hours during which ETx was infused.

THERAPEUTIC IMPLICATIONS

Emerging evidence about the effects of LeTx and ETx raises several issues and questions regarding the conventional treatment of *B. anthracis*-related shock. Although antibiotics are clearly important in management of *B. anthracis*, their efficacy might not only be due to bacterial clearance. It is possible that agents such as clindamycin or rifampin, used in the 2001 outbreak, might also inhibit protein or RNA synthesis necessary for toxin production (65). Later in infection, however, when toxin levels are likely the highest, the immunosuppressive effects of both LeTx and ETx could inhibit microbial clearance and impair the efficacy of antibiotics. It is noteworthy that, in the absence of antibiotics, PA-monovalent antibody (PA-mAb) treatment in a spore-challenged rabbit model was associated with the clearance of bacteria from blood cultures compared with placebo-treated animals (66).

It is also currently unknown how the vascular effects of LeTx and ETx might alter responsiveness to conventional hemodynamic support. As noted, the recurrent pleural fluid accumulation and marked hemoconcentration observed in patients with *B. anthracis* are consistent with emerging data regarding abnormalities of endothelial cell function associated with LeTx as well as the original effects associated with ETx on local edema formation (1, 4, 7, 38–41). In this context, whether standard volume support is as efficacious during *B. anthracis* infection as it is during other types of sepsis is unknown. In our rat model, in contrast to *E. coli* and LPS challenge, fluid support worsened outcome with LeTx challenge (6). Furthermore, review of all reported inhalational anthrax cases over the past 100 years has suggested that aggressive and frequent drainage of the pleural effusions with *B. anthracis* may be associated with improved outcome (1). Whether LeTx and ETx are also capable of altering the response to conventional vasopressor treatments is unknown. Certainly, disruption of intracellular calcium metabolism by the adenylyl cyclase activity of ETx could alter the effects of such therapy (53, 56, 60). However, whether any one vasopressor is more effective than others for shock with LeTx and ETx requires study.

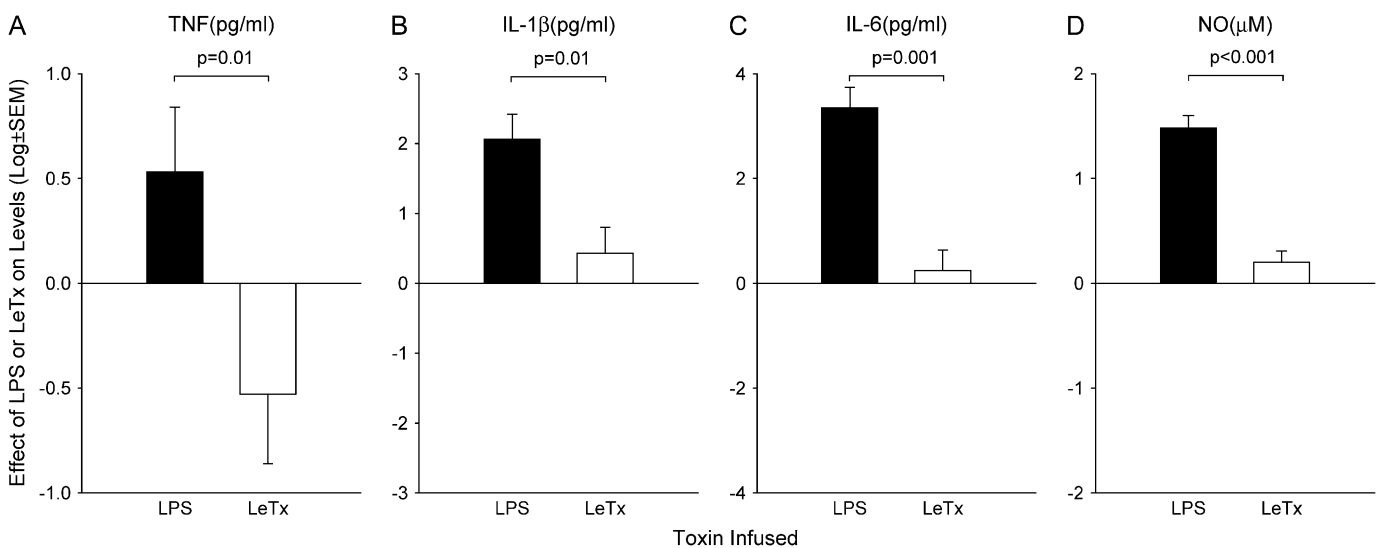


Figure 4. A comparison of the effects of similarly lethal 24-h infusions of lipopolysaccharide (LPS) or LeTx on mean serum (A) tumor necrosis factor (TNF), (B) interleukin (IL)-1 β , (C) IL-6, and (D) nitric oxide (NO) levels measured from 3 to 9 h. Control animals (data not shown) were challenged with diluent only. Compared with LPS, LeTx was associated with greatly reduced measures of each of these mediators and TNF levels were actually reversed (28).

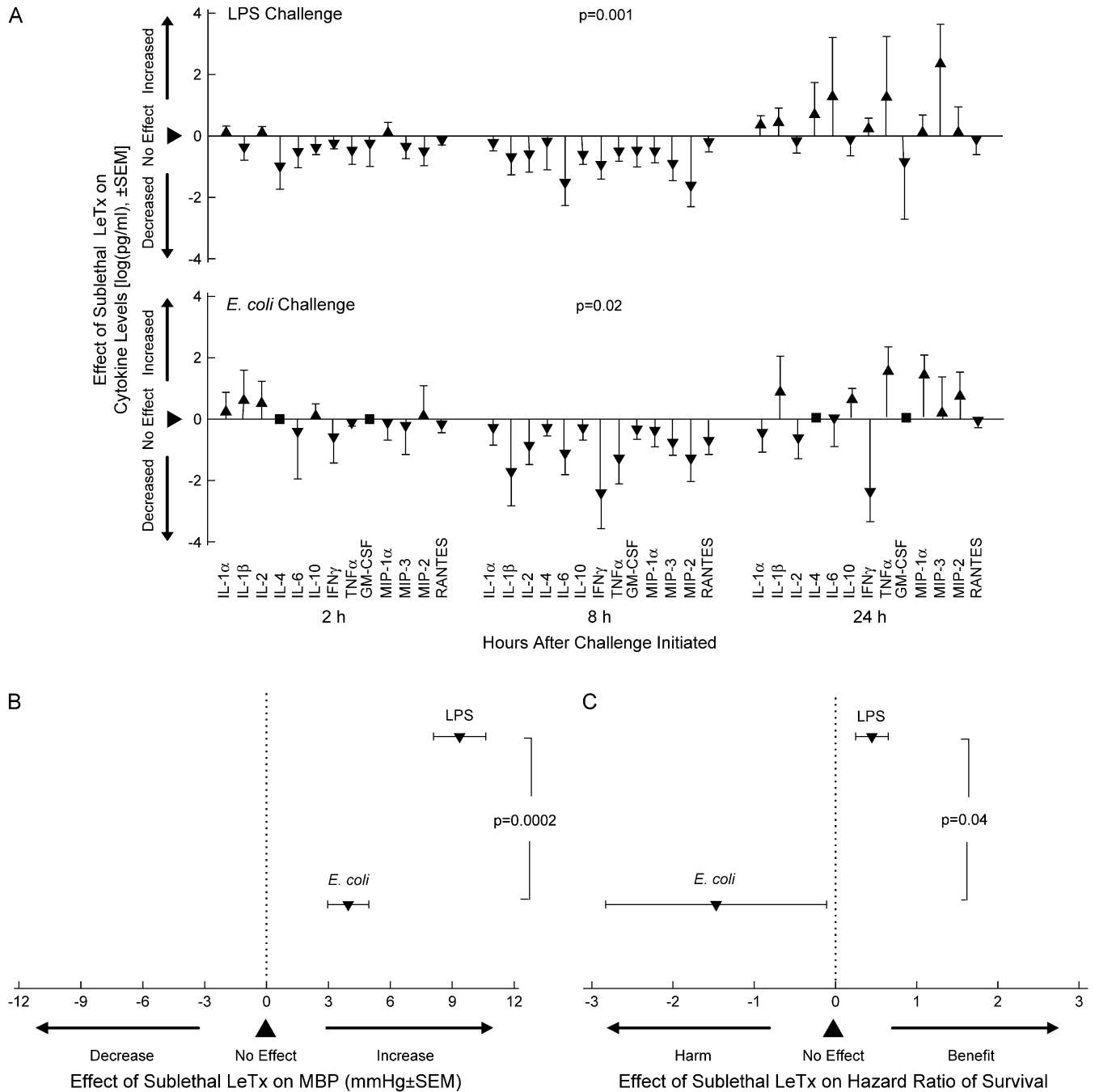


Figure 5. The effects of sublethal doses of LeTx compared with placebo administered 3 h before intravenous LPS or intratracheal *E. coli* challenge on (A) 13 different plasma cytokine levels, (B) MBP, and (C) the odds ratio of survival. Cytokines were measured at 2, 8, and 24 h; blood pressure was averaged over the 24 h after challenge; and survival was assessed after 168 h. With both LPS and *E. coli*, sublethal LeTx had variable effects on cytokines at 2 and 24 h but consistently reduced all 13 levels at 8 h. The consistent decreases caused by LeTx in all cytokines at 8 h was significantly different from the more variable changes noted at 2 and 24 h with both challenges ($p = 0.001$ and 0.02 as shown in A). These antiinflammatory effects of sublethal LeTx, while increasing MBP and survival with LPS challenge, produced smaller MBP increases and reduced survival with *E. coli* in patterns that were significantly different comparing the two challenges (37).

Two other therapies used with increasing frequency clinically during sepsis deserve mention (67). Inhibition of GR function by LeTx raises the possibility that corticosteroid therapy might be beneficial during shock with *B. anthracis* (42). However, in the limited studies conducted to date, dexamethasone administration was not effective in LeTx-challenged animals models (42). Finally, although not clearly attributable to LeTx or ETx,

the mediastinal hemorrhagic necrosis and recurrent hemorrhagic pleural effusions noted in patients with *B. anthracis* may make the use of recombinant human activated protein C, with its associated bleeding risks, problematic (68).

Despite such questions, growing knowledge of the structure and function of both LeTx and ETx has led to the design of an increasing number of agents capable of neutralizing these toxins

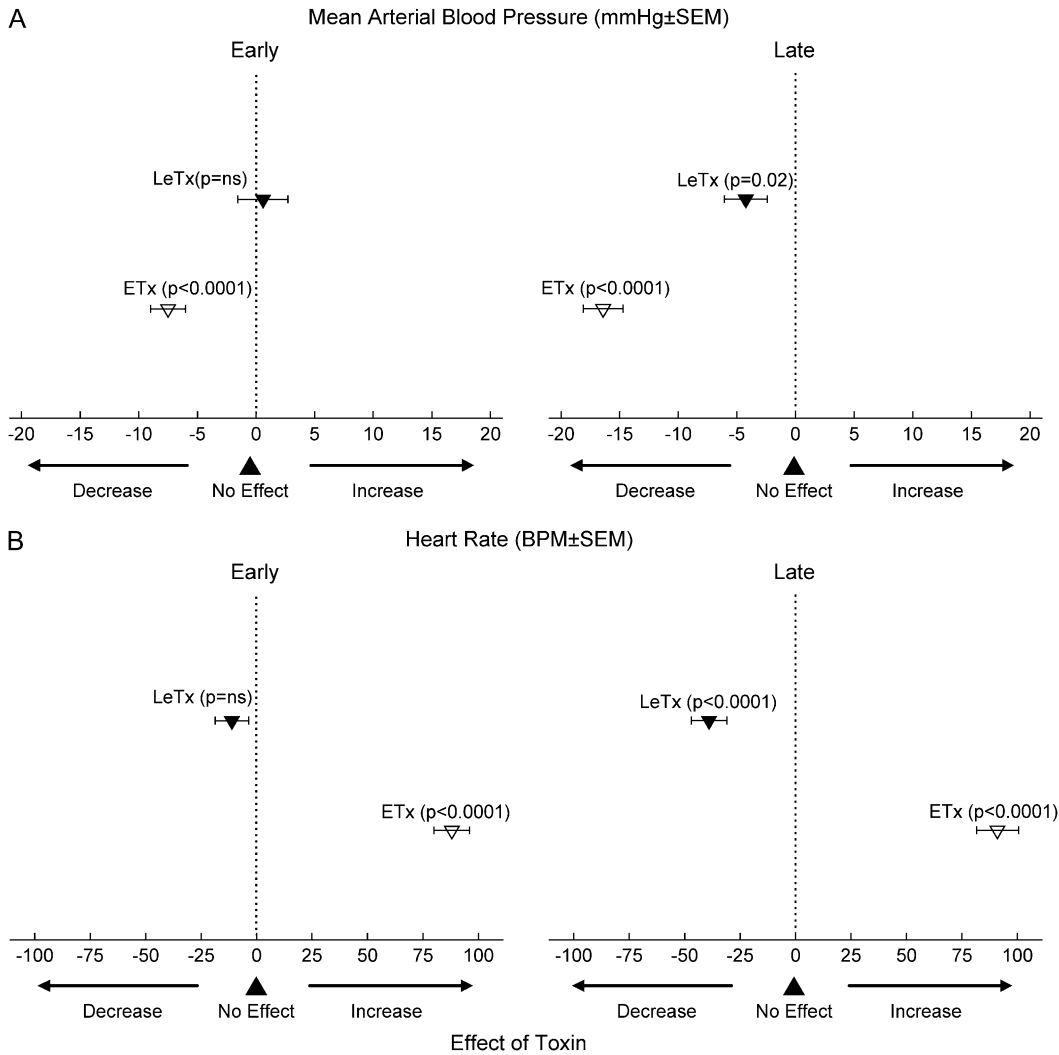


Figure 6. The effects of similarly lethal 24-h infusions of LeTx and ETx in rats on (A) MBP and (B) heart rate, early (2 to 10 h) and late (12 to 24 h), compared with animals receiving diluent only (data not shown). ETx produced earlier and greater reductions in MBP and increased rather than decreased heart rate compared with LeTx (59).

or their effects (Figure 1). Even before the availability of effective antibiotics, antiserum developed against *B. anthracis* was used in the acute treatment of patients with inhalational anthrax and, in some reports, may have prevented death (1). Such agents may be even more important because antibiotics, although quickly clearing bacteremia in patients during the 2001 outbreak, still did not prevent lethality in some (69). Agents inhibiting PA may have an advantage because they block both LF and EF. Antibodies against PA have been obtained from animal and human sources and humanized mAbs have been synthesized (70–77). PA antibodies prevent toxin entry into cells and macrophage lysis (73). These antibodies have been shown to be effective after the onset of shock with LeTx challenge and established infection with spore challenge (29, 70). PA antibodies appear to be safe in human volunteers (72). On the basis of such data, the U.S. military has now purchased 20,000 doses of a PA-directed mAb (Human Genome Sciences, Rockville, MD) (78). In addition, the U.S. Department of Health and Human Services is acquiring 10,000 doses of anthrax immune globulin (79). Animal data suggest, however, that antibodies like these will be most effective if administered early after shock is recognized (29).

Several other treatments directed against PA have also appeared to be effective in *in vitro* or *in vivo* models. Mutant

forms of the PA molecule (dominant-negative inhibitors) with abnormal $2\beta 2-2\beta 3$ loops that form dysfunctional heptamers with wild-type PA prevented the uptake and transport of LF or EF and were effective with LeTx challenge in cell culture and Fisher 344 rats (80, 81). Hexa-D-arginine, a furin inhibitor that blocks PA cleavage and heptamer formation, was protective with LeTx challenge in murine alveolar macrophages and Fisher 344 rats (82). Finally, soluble PA receptor protected against LeTx in *in vitro* studies (13).

Agents have also been developed to directly inhibit LF. An mAb (LF8) directed against the PA binding site of LF was protective in macrophage and mouse models with LeTx (83). An antibody to domain III of LF prevented macrophage lysis *in vitro* and was protective in LeTx-challenged Fisher 344 rats (84). Small-molecule inhibitors of LF have been identified by chemical screening, including one that was effective with spore challenge when added to ciprofloxacin (85–87). Polyphenols from green tea, such as catechin, were protective with LeTx challenge *in vitro* and in rats (88). Finally, peptide molecules that act as substrate inhibitors of LF metalloproteolytic activity were protective *in vitro* (89, 90).

Treatments against EF have also been identified and may be useful if combined with anti-LF treatments. Adefovir, a drug used for chronic hepatitis B infection, inhibited EF with great

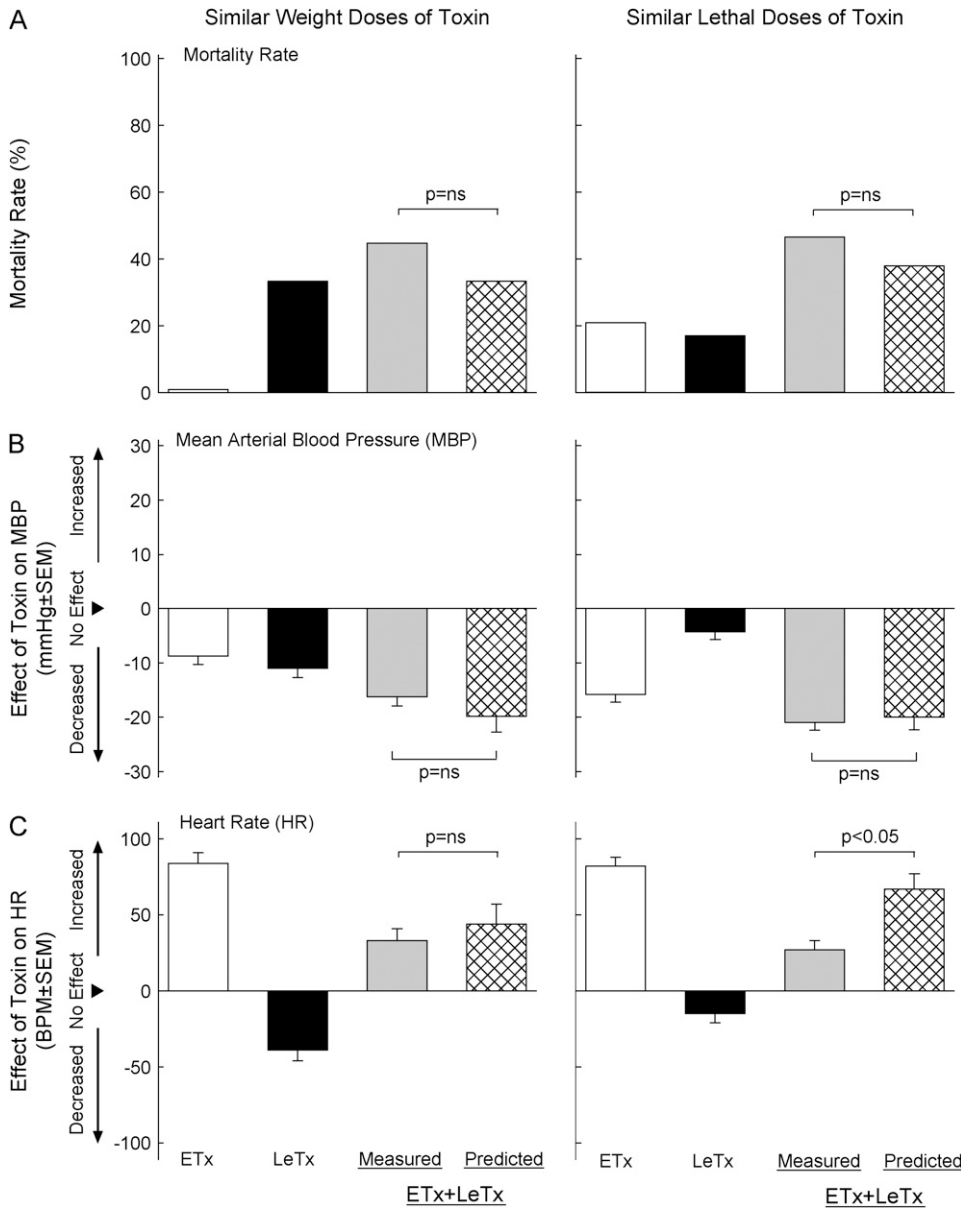


Figure 7. The effects in rats on (A) survival, (B) MBP, and (C) heart rate (HR) of 24-h infusions of ETx and LeTx alone or together using doses that were similar either in weight or lethality. Control animals received diluent only. Changes in MBP and HR were averaged from 12 to 24 h during the infusions and survival was measured at 168 h. The two toxins together, whether in doses similar in weight or lethality, increased mortality rates and reduced MBP in patterns not different from the ones predicted based on their effects alone. Likewise, the effects of the toxins together on HR were always intermediate between their effects alone. Thus, ETx and LeTx appeared to have additive effects in this model (59).

affinity in murine macrophages and prevented EF-induced increases in cAMP (91). A quinazoline selectively inhibited EF without affecting mammalian adenyl cyclases (92).

CONCLUSIONS AND SUGGESTIONS FOR FUTURE RESEARCH

There has been considerable progress toward understanding the structure and function of LeTx and ETx and their possible contributions to shock with *B. anthracis*. At this time, however, important questions remain regarding the precise nature and mechanisms underlying the cardiovascular dysfunction these toxins produce. For example, the extent to which peripheral vascular dysfunction as opposed to myocardial injury contributes to shock with these toxins requires investigation. Furthermore, the question of how responsive this dysfunction is to conventional support has received little attention. Clearly defining the effects of fluid support both with toxin and live bacterial infection is essential at this time as is clarifying the effectiveness of conventional

vasopressor support with agents such as norepinephrine and vasopressin. It is increasingly clear, however, that these toxins may have substantial suppressive effects on normal host defense function. Investigating whether these suppressive effects participate in the establishment of infection or contribute to the high bacterial loads noted in patients dying of *B. anthracis* will be important. It will also be necessary to determine whether other components of *B. anthracis* besides LeTx and ETx contribute to the shock occurring with infection. In light of the homology of TEM8 and CMG2 to von Willebrand factor, whether the interaction of these receptors with PA contributes to endothelial injury and to even the disseminated intravascular coagulation that has been observed during *B. anthracis* infection in humans and animals requires study (69, 93). Encouragingly, recent work directed at LeTx and ETx has resulted in a range of new agents capable of reducing their injurious effects. Understanding how best to apply these agents and how they interact with conventional treatments should be additional goals for future research.

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