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MINIREVIEW

Choice of Bacteria in Animal Models of Sepsis

ALAN S. CROSS,[1]* STEVEN M. OPAL,[2] JERALD C. SADOFF,[1] AND PETER GEMSKI[3]

Divisions of Communicable Diseases and Immunology[1] and of Pathology,[3] Walter Reed Army Institute of Research, Washington, D.C. 20307-5100, and Department of Medicine, Brown University School of Medicine, Providence, Rhode Island 02860[2]

INTRODUCTION

With the recognition of the paramount role of inflammatory mediators in the pathogenesis of sepsis, there has been a rapid profusion of new reagents and strategies for the treatment of bacterial sepsis. The dynamic nature of the pathophysiologc events and the complex array of mediators generated in the septic process preclude accurate prediction of the efficacy of novel therapeutic agents from in vitro studies alone. Experimental data derived from animal research have been and will continue to be indispensable in the development of new therapeutic strategies for sepsis. The use of animal models of sepsis can provide invaluable information on the safety, efficacy, and pharmacokinetics of immunotherapeutic agents.

Endotoxin, or lipopolysaccharide (LPS), has a central role in the initiation of the septic process. Circulating endotoxin in the absence of bacteria or circulating endotoxin liberated from growing or antibiotic-killed bacteria has been measured in association with sepsis (5, 15, 55, 65). Consequently, putative modulators of the sepsis syndrome are often evaluated for their ability to attenuate the effects of LPS administration to animals (3, 28). Following the assessment of a modulator's effect after endotoxin administration, it often is next tested for its ability to alter the course of sepsis following the administration of live bacteria to the animals. The intent of such challenges with live microbes is to stimulate the conditions in which bacterial infection leads to sepsis (1, 22, 27, 29, 49, 57, 62). Thus, the literature on sepsis distinguishes between two types of animal models of sepsis: endotoxin challenge and infection. Examination of this literature further reveals that some of the animal models of sepsis following challenge with very high doses of live organisms may more appropriately reflect intoxications similar to those resulting from LPS challenge. Typically in such experiments, the animal host is only responsive to the microbe at very high doses and the pathogen does not colonize and replicate significantly following challenge. Hence, the cytokine responses by the animal host to such insult may not be typical of those measured during true sepsis in animals and humans that progresses from a focus of infection.

Since animal species differ considerably in their cardiovascular physiology and susceptibility to bacterial endotoxin, investigators carefully consider the relative merits and limitations of each animal model before extrapolating animal data to clinical efficacy in septic patients. Having given such consideration, however, many investigators do not give sufficient thought to the choice of the bacterial strain for use in these animal models. It is our contention that the transient introduction of high doses of bacteria into an animal so as to establish high bacteremia counts does not necessarily represent a reliable sepsis model for evaluating the toxicity, efficacy, and safety of immunotherapies. Rather, more realistic animal models of sepsis necessitate carefully choosing an experimental host capable of being efficiently infected at relatively low doses with an appropriate pathogen and demonstrating that such initial colonization progresses to sepsis.

While previously there have been widely varying definitions of sepsis, there is a growing consensus that sepsis might be defined as a clinical syndrome characterized by a systemic physiologic response, including organ hypoperfusion and dysfunction, mediated by endogenous modulators whose activity may be initiated by a wide variety of stimuli. Untreated, the sepsis syndrome may progress to multiorgan failure and death. Recent large-scale studies have found that gram-negative bacilli, the focus of this minireview, account for approximately 40% of cases of sepsis (4, 25, 67, 76). While few animal models completely simulate the sepsis syndrome in humans, models that permit an evolution of sepsis from a focus of infection rather than utilizing a fulminant, short-lived course following the bolus administration of relatively avirulent organisms more accurately reflect the course of human sepsis.

BACTERIAL SURFACES AS DETERMINANTS OF VIRULENCE

It is now well established that microbes have evolved a broad array of structural and soluble components that are important participants in the pathogenesis of infections. For the purpose of this review, we shall selectively address those bacterial properties that serve as recognized factors that facilitate evasion of host defenses and potentiate the development of sepsis.

Nearly 40 years ago, Rowley experimentally defined a virulent strain of *Escherichia coli* as one that was able to grow in vivo from a small inoculum (53). To replicate within an animal host, the organism must possess the capability to evade host defenses and to acquire essential nutrients in this hostile environment. Other investigators have since added that a successful pathogen must also have the ability to invade host tissues and disseminate (38, 59).

Earlier experimental studies of complement-mediated bacteriolysis have established that the evasion of this host defense mechanism is a property of the pathogen and not of the host (71). Bacteria resistant to complement-mediated bacteriolysis must express proper surface characteristics, such as a complete or smooth LPS (10, 37, 38, 41, 45, 50, 54)

* Corresponding author.

or an acid polysaccharide capsule, or K antigen (10, 24, 30, 40, 45). Bacteria having either a smooth LPS but lacking a capsule or rough LPS in the presence of a capsule are each capable of resisting serum bacteriolysis. Indeed, such importance of the capsule as a prerequisite for virulence of *E. coli* was recognized by Smith and Little over 60 years ago (60).

The phase of growth is a critical factor in the surface expression of both complete O side chain and capsular polysaccharide. Bacteria harvested in log phase have more complete LPS and a higher content of capsular polysaccharide than bacteria grown to stationary phase (66). Consequently, even for bacteria with the requisite surface properties, the phase of growth may importantly affect the behavior of the bacterial strain in both in vivo (50% lethal dose [LD_{50}]) and in vitro (serum bactericidal) assays of virulence (2, 66).

Serum resistance was found to be an important characteristic not only of bacteria used in experimental studies of sepsis but also of strains cultured from human blood. Repeatedly it has been observed that the preponderance of clinical bacteremic isolates resist the bacteriolytic activity of serum (10, 36, 51, 52).

Despite such a serum-resistant phenotype, most bacteremic isolates can be susceptible to opsonophagocytic killing by neutrophils in the presence of fresh human serum (10). With *E. coli* pathogens, the important contributions of LPS and capsular polysaccharide to evasion not only of complement-mediated bacteriolysis but also of neutrophil-mediated opsonophagocytosis have been well delineated (10, 37, 38).

In the case of *E. coli* with the K1, K5, and perhaps other K antigens, the capsular structures appear to chemically mimic host cell determinants. Thus, these bacteria are able to evade both host immune surveillance and subsequent antibody response. Bacteria that express such K antigens as well as a smooth LPS phenotype may be particularly capable of evading both complement-mediated and neutrophil-mediated host defenses. For these select phenotypes, cytokine-activated mononuclear cells are required for bacterial killing. We, and others, have shown that cytokines, such as tumor necrosis factor (TNF) and interleukin-1 (IL-1), are important mediators that function in host defense mechanisms against not only known intracellular pathogens but also bacteria that may cause sepsis (12, 14, 42, 46).

The relative roles of these surface determinants in the pathogenic process are reflected in both in vitro (serum bactericidal and opsonophagocytic assays) and in vivo (LD_{50}) studies of pathogens. For example, *E. coli* K-12, a standard laboratory strain which lacks a polysaccharide capsule and has a rough LPS phenotype, is lysed by as little as 10% fresh normal human serum (NHS). It is unlikely to survive in the blood long enough to encounter a phagocyte. For comparison, a common isolate from neonatal meningitis or adult bacteremia, *E. coli* O18:K1:H7, is not killed by as much as 80% NHS and is poorly killed by NHS and neutrophils in the absence of specific antibody (13). Thus, in the absence of such surface determinants, bacteria are efficiently cleared by the host and the bacteria are unable to establish an infection. As a consequence, for such relatively avirulent bacteria to elicit a cytokine response, one must administer an inoculum sufficiently large to overwhelm, or intoxicate, host defenses. However, as will be discussed below, the cytokine response induced in animals by the infusion of such bacteria differs significantly from the septic response in humans in both peak levels achieved and the duration of that response.

The study of the seroepidemiology of bacteremic *E. coli* has also confirmed the importance of specific surface determinants in extraintestinal infection. It has been recognized for nearly a half century that only a relatively limited number of O serogroups (e.g., O1, O2, O6, O7, O6, O8, O16, O18, O25, and O75) or K antigens (K1, K2, K5, and K12) of *E. coli* have the necessary characteristics that permit the bacteria to survive and disseminate outside the intestine (10, 32, 36, 44, 45, 58, 63). These limited number of strains, or potential clones, have consistently been isolated from clinical bacteremic and meningitic *E. coli* infections worldwide (9). Of the greater than 100 K antigens, the K1 capsule alone is found in over 20% of bacteremic isolates of *E. coli*, and only 6 of these 100 K antigens are found in the majority of cases of bacteremia. Of the greater than 170 O serogroups, only 10 to 12 are commonly found during bacteremia. These serologic groups may represent a few types of *E. coli* whose surface chemical structures have evolved sufficiently to impart to the organisms the ability to evade host defenses and become invasive pathogens. For example, the sialic capsule of K1 *E. coli* favors the deposition of serum factor H over factor B (20). The chemical structures of most capsular chemotypes favor the deposition of factor B and the amplification of a complement complex forming on the bacterial surface for the purpose of converting complement protein 3 into an opsonin. In the case of the sialic acid of the K1 capsule, the binding of factor H is favored and this hastens the degradation of this nascent complex, thereby limiting the deposition of the C3b and C3bi opsonins on the bacterial surface. This permits the organism to avoid uptake and killing by phagocytes.

E. COLI O86 and O111 LACK AN EXTRAINTESTINALLY INVASIVE PHENOTYPE

Many strains of *E. coli* used in the past as live bacterial challenges in models of sepsis, such as the baboon model, have not been associated with extraintestinal, disseminating sepsis. Included among these challenge organisms are *E. coli* serogroups O86 and O111, which previously have been identified as classic enteropathogenic *E. coli* that caused outbreaks of diarrhea in newborn nurseries shortly after World War II (26). They have been extensively characterized experimentally and have been found to be avirulent in nondiarrheal models (21, 37, 38, 53). When fed orally to adult human volunteers, inocula in excess of 10^8 CFU caused minimal intestinal symptoms and no bacteremia (21). Our studies have shown that O86 and O111 strains, known to be unencapsulated, express LPS phenotypes which are part rough and smooth, respectively, based on susceptibility to rough LPS-specific bacteriophages and the presence of a typical ladder appearance on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (10). Each is efficiently killed by serum (nearly 100% by 10% NHS in 60 min) in the absence of neutrophils. Consequently, the ease with which *E. coli* O111:B4 is killed by complement illustrates that not only smooth LPS phenotype but also LPS subunit composition are critical determinants of serum resistance (47).

Such characteristics associated with O86 and O111 strains likely account for their avirulence in animal models. The LD_{50}s of these isolates in mice are $>1 \times 10^8$ CFU. Hence, they are more than one million-fold less virulent than *E. coli* O18:K1, a human pathogen known to cause bacteremia in adults and meningitis in neonates (8, 36a). These strains are so efficiently killed, probably by the complement system alone, that an infusion of $>10^{10}$ CFU is generally required to

TABLE 1. Levels of TNF in the blood of human volunteers following the experimental infusion of endotoxin

Reference (yr)	No. of subjects	Endotoxin dose	Maximum TNF level (pg/ml) (range)	Assay[a]	Time (min) to maximum	Time (h) to barely detectable TNF
27 (1988)	4	20 U/kg	358 ± 166 (NS[b])	E	90	2.5
39 (1988)	13	4 ng/kg	240 ± 70 (35–730)	E	90–180	4.0
7 (1990)	6	4 ng/kg	ca. 750 (NS–ca. 600)[c]	RIA	90	<250 pg/ml at 4.0
64 (1990)	6	2 ng/kg	550 (68–1,374)	RIA	60–90	<10 pg/ml by 6.0
34 (1991)	6	4 ng/kg	812 ± 331 (ca. 500–1200)[c]	E/B	90	<15 pg/ml at 5.0
61 (1991)	7	NS	369 ± 44 (245–570)	E	90	Baseline at 6.0

[a] E, enzyme-linked immunosorbent assay; RIA, radioimmunoassay; B, bioassay.
[b] NS, not stated.
[c] ca. indicates that value was obtained from figure. No exact value provided in text.

induce any systemic response (27, 49, 56, 57). Even at this bacterial load, there is rapid clearance of circulating bacteria within 60 min of cessation of the infusion (48). These strains therefore do not behave as typical virulent extraintestinal pathogens as defined by either Rowley or Smith (53, 59): (i) they are unable to grow in vivo from a small inoculum and disseminate, (ii) they cannot evade host defenses, and (iii) they cannot cause extraintestinal infection. Indeed, these strains are rarely, if ever, reported as extraintestinally invasive, even in immunocompromised hosts. In one recent report of an outbreak of diarrhea caused by *E. coli* O111:B4 that involved over 100 cases, there was not one case of bacteremia reported (68).

ANIMAL MODELS OF INFECTION AND INTOXICATION INDUCE DIFFERENT CYTOKINE KINETICS

The infusion of a large bolus of live bacteria (10^{11} to 10^{12} CFU) into animals represents an effort by investigators to overwhelm host defenses and to establish a condition of sepsis. It is more appropriate to recognize that such an experimental protocol establishes an intoxication rather than sepsis. The load of endotoxin contained in 10^{12} *E. coli* CFU is approximately 1 to 2 mg of purified LPS (see Appendix). When directly compared in mice, there was little difference in the physiological responses of animals to a bolus infusion of a large dose of live *E. coli* O111 and to the same dose of heat-killed bacteria (57). Clinical experience, however, has shown that during bacteremia colony counts rarely exceed 10^4 CFU/ml of blood (18, 72), with 10^7 CFU/ml being the highest bacterial concentration in blood reported (55).

Similarly, there are significant differences between the levels of endotoxin measured in the blood of patients with sepsis and of animals given a large bolus of bacteria. Circulating endotoxin may not be detected in up to half the cases of clinical sepsis (19, 74). When present, endotoxin loads in the low to mid-picogram range typify conditions of clinical bacteremia (55). During nonmeningococcal sepsis, mean levels of circulating endotoxin in the range of 180 (8) to 440 (15) pg/ml have been reported. These levels are greatly increased during meningococcal sepsis, in which levels of >700 pg/ml have been associated with shock and 3,800 pg/ml has been associated with a fulminant course (5). In contrast, a peak level of endotoxin of 11,800 pg/ml was detected in the blood of baboons following an infusion of live bacteria over 8 h (49).

Several studies have now emphasized that distinctions in cytokine responses were evident when LPS intoxication and gram-negative clinical sepsis were evaluated in humans. In comparing both the level and duration of cytokine production after endotoxin challenge (Table 1) and during clinical sepsis (Table 2), there is a significant high peak level of TNF observed in all volunteers following the infusion of endotoxin. This cytokine production is of short duration (<6 h), however. In contrast, TNF is found in approximately half of the patients diagnosed with sepsis (Table 2). The peak level of circulating TNF in patients with detectable cytokine is a fraction of that measured after experimental LPS infusion in humans. In two studies of meningococcal sepsis (69, 70), the TNF levels of one patient with outlying data increased the mean levels for all patients from 92 ± 102 to 1,588 ± 6,349 pg/ml (70) and from 128 ± 199 to 3,115 ± 9,448 pg/ml (69). Unlike what occurs in experimental studies, circulating TNF can be found for over 7 days after initial diagnosis when blood samples are obtained (6, 8). These distinct kinetic responses may be attributable to the rapid clearance and detoxification of the endotoxin after its infusion on the one hand (35) and to the lower but persistent presence of the bacterial stimulus during sepsis on the other. Unlike the temporally limited intravenous presentation of LPS to experimental animals, endotoxin is generally presented to the septic patient by progressive translocation of viable gram-negative bacteria across mucous membranes or as a consequence of bloodstream invasion from a localized infection.

The infusion of live avirulent bacteria into animal models of sepsis (Table 3) induces TNF kinetics similar to that observed with endotoxin challenge and markedly different from that observed in septic patients. The high peak levels of TNF following intravenous infusion of these high doses of bacteria compared with the lower levels of circulating bacteria in clinical disease are consistent with the experimental observation that there is a correlation between the concentration of bacteria to which the macrophages are exposed and the induction of TNF (31). The rapid clearance of the bacteria used in these studies parallels that of endotoxin in the studies cited in Table 1 and confirms their relative inability to establish extraintestinal infection (73). In contrast, the use of strains known to cause sepsis in humans induced in animals a peak level of TNF of approximately 500 pg/ml that was detectable for an extended time (11, 43). This differing pattern of cytokine release between intoxication and infection models is evident even when the same strain of *E. coli* is administered intravenously as a bolus infusion inducing transient bacteremia (i.e., an intoxication) or intraperitoneally as an ongoing infection. Peak serum cytokine levels were 50 to 100 times greater with bacterial challenge by the intravenous route than by the intraperitoneal route; however, the cytokine levels were sustained during the ongoing peritoneal infection (75).

TABLE 2. Levels of TNF in the blood of patients diagnosed with sepsis

Reference (yr)	No. of patients with TNF/ total no.	Mean TNF level at time of diagnosis of sepsis (pg/ml) (range)	Assay[a]	Duration	Comments
70 (1987)	18/79	1,588 ± 6,349 (2–27,020)	B	NS[b]	In 17/18, mean TNF of 92 ± 102 pg/ml
23 (1988)	32/35	NS (250–1,300)	RIA	NS	Children mostly with meningococcal purpura; at diagnosis, nonsurvivors had 830 ± 340 pg/ml
16 (1989)	11/43	42 ± 31 (15–122)	E	NS	No TNF in 7 patients with gram-negative bacteremia
69 (1989)	10/10	3,115 ± 9,448 (7–30,000)	B	Up to 18 h	All with meningococcal sepsis; in 9/10 mean TNF of 128 ± 199 pg/ml
17 (1989)	6/38	202 ± 159 (50–500)	E	Up to 48 h	TNF level not predictive of complications
8 (1990)	NS/97	93 (0–1,000)	E	Up to 7 days	36% bacteremic; TNF not predictive of poor outcome
33 (1990)	27/74	167 ± 53	E	Decreased at 24 h	No difference in TNF levels in gram-positive or -negative bacteremia
6 (1990)	55/70	180 for survivors; 330 for nonsurvivors	RIA	Up to 10 days	At day 10, TNF reduced in survivors to <100 pg/ml and in nonsurvivors to 305 pg/ml
19 (1992)	18/19	122 (3–4,167)	RIA	Up to 24 h	TNF reduced in survivors by 122 pg/ml and in nonsurvivors by 27 pg/ml
74 (1992)	NS/65	27–35 (5–2,264)	RIA	NS	No decrease at 24 h in in placebo group

[a] B, bioassay; E, enzyme-linked immunosorbent assay; RIA, radioimmunoassay.
[b] NS, not stated.

DIFFERING IMPLICATIONS OF INTOXICATION VERSUS INFECTION MODEL USE

Distinctions between models of intoxication and infection must be made in order to properly evaluate either the true efficacy or potential toxicity of treatment regimens for sepsis. For example, in an assessment of efficacy, antibody to TNF prevented lethal sepsis in primates when given prophylactically but not when given as treatment following endotoxin challenge (3). In another study, a monoclonal antibody (MAb) against TNF had to be given at least 2 h before a lethal infusion of bacteria to prevent death (62). In contrast, in another (infection) model of sepsis a different MAb to TNF was effective when given after bacterial challenge as treatment (11, 43). If one makes the assumption of a quantitative relationship between the amount of TNF present and the amount of anti-TNF antibody required for its neutralization, then there may be a reasonable explanation for these conflicting results. In the first study, anti-TNF MAb could neutralize the cytokine as it was being produced following stimulation but when given following doses of bacteria that induced hyperproduction of TNF (peak level of 21,500 ± 3,800 pg/ml), there may have been a clearly insufficient amount of antibody to neutralize the TNF. In the infection model, on the other hand, anti-TNF MAb at a dose of 20 mg/kg was effective as treatment because there was an amount of circulating TNF (18.9 ± 3.4 U/ml, or approximately 200 pg/ml) at the time anti-TNF MAb treatment was initiated that was similar to those found in humans with sepsis. The latter level of circulating TNF is closer to what has been reported for human sepsis (Table 3).

Animal models of infection are also essential for evaluating any potential toxicities of antimediator therapy. Unlike

TABLE 3. Levels of TNF in the blood of animals following the infusion of live bacteria

Reference (yr)	Animal	Bacterial dose[a]	Mean peak TNF level (pg/ml)	Assay[b]	Time (h) to peak	Time (h) to baseline
62 (1987)	Baboon	EC, 1 LD_{100} i.a., 0.5 h	21,500 ± 3,800	E	1.5–2.5	4–6
29 (1990)	Baboon	EC, 5 × 10^{10} CFU/kg i.v. over 2 h	148,000 ± 66,000	B	2.0	4–6
27 (1988)	Baboon	EC O86, 10^{11-12} CFU/kg i.v. over 40 min	20,500 ± 9,890	E	1.5	Within 6
49 (1991)	Baboon	EC O86, 10^{10} CFU/kg i.v. over 8 h	730 ± 300	RIA	1.0	Detectable at end of 8-h infusion
57 (1990)	Mouse	EC O111, 9 × 10^8 CFU/kg	232,000 ± 39,000	E	0.8	12,000 pg/ml at 5
		PA, 2 × 10^8 CFU/kg	62,000 ± 28,000	E	1.5	1,300 pg/ml at 5
		KP, 10^9 CFU/kg	49,700 ± 5,000	E	1.5	660 pg/ml at 5
56 (1990)	Mouse	EC O111, 9 × 10^8 CFU i.v. bolus	180,000	E	1.5	5
1 (1991)	Rat	EC (E11775)[c]		B		
		10^{10} CFU i.v.	30,000 U/ml		1–2	4
		10^7 CFU i.p.	ca. 150 U/ml		2.0	7

[a] EC, *Escherichia coli*; KP, *Klebsiella pneumoniae*; PA, *Pseudomonas aeruginosa*; i.a., intraaortic; i.v., intravenous; i.p., intraperitoneal. No serotype provided for references 29 or 62.
[b] E, enzyme-linked immunosorbent assay; RIA, radioimmunoassay; B, bioassay.
[c] Urinary tract isolate.

antibiotics that achieve relatively predictable levels in the body and then are excreted with relatively little effect on host defenses, the current candidates for septic therapy are potent immunomodulators that are capable of altering host defenses to either the benefit or the detriment of a patient.

There is growing evidence that cytokines, such as TNF and IL-1, impact host defenses against a broad array of microbial pathogens. For example, the experimental use of anti-TNF antibody in both salmonella and listerial infection induced a lethal worsening of those infections (42). Conversely, the administration of exogenous recombinant TNF and IL-1 in nanogram doses protected C3H/HeJ mice from lethal infection with *E. coli* (12). Simply doubling the dose of IL-1 reversed this beneficial effect, perhaps by the induction of known positive feedback loops for cytokine production. Thus, there appears to be a well-controlled orchestration of the cytokine response, and relatively minor modifications of cytokine physiology, either in levels or temporal sequence, might improve or worsen the outcome of infection. From this perspective, the sepsis syndrome might not be so much an abnormal host response to infection as a dysregulation of a normal host response. Therefore, the appropriate question may not be whether TNF, interferon, or IL-6 is the more important mediator of septic shock but rather under what conditions and by what mechanisms does the loss of regulation of these cytokines occur? Consequently, it is anticipated that modulation of these cytokine responses might result in a beneficial outcome in some clinical situations, such as gram-negative bacteremia with shock, in which there is an overflow of cytokines into the general circulation, and in a deleterious outcome in others, such as gram-negative infection without shock, in which the cytokine regulatory networks are still intact. The provision of anti-TNF MAb might in some situations be considered to be similar to arming a potential pathogen with an additional virulence determinant that it may use to evade critical host defense mechanisms. In contrast, the administration of anti-TNF MAb in the presence of *E. coli* K-12, O111:B4, or O86, strains that do not require the host to utilize a defense mechanism any more primordial than circulating complement, might not permit detection of the potential detrimental effects of such immunomodulatory therapy.

In order to predict such results, both in terms of efficacy or potential adverse events, one must use a true infection model, not an intoxication model. This demands the choice of appropriate challenge strains of bacteria. The British poet Pope asserted that the proper study of mankind is man. Were he alive today and studying sepsis, he might well have added, "For the proper study of sepsis, study bacteria that cause sepsis."

APPENDIX

E. coli O18:K1:H7, strain Bort, was grown to mid-log phase, washed, and adjusted spectrophotometrically to an optical density of 2.0 (at 650 nm), corresponding to a bacterial concentration of approximately 10^{10} CFU/ml. Serial 10-fold dilutions were made in nonpyrogenic normal saline, and determinations were made of *Limulus* lysate reactivity in a chromogenic assay (Bio-Whittaker, Walkersville, Md.) according to the manufacturer's recommendations. Readings of *Limulus* reactivity were made for each bacterial dilution and correlated to an endotoxin standard curve provided in the assay kits. Postdetermination bacterial colony counts were made to confirm the inoculum added at each dilution. A bacterial inoculum of 1.6×10^5 CFU had 2.055 endotoxin units (EU), 1.6×10^4 CFU had 0.468 EU, 1.6×10^3 CFU had 0.049 EU, and 1.6×10^2 CFU had 0.001 EU. Based on the manufacturer's estimation of approximately 84 pg/EU, there would be approx. 1 to 2 mg of endotoxin in the 10^{12}-CFU inoculum. Since LPS preparations may be considerably more active than standard LPS preparations provided in the commercial kits, this may be a conservative estimate of endotoxic activity in this inoculum. Alternatively, lyophililzation of 1.15×10^{12} CFU of *E. coli* yielded a dry weight of 268 mg. If one uses a conservative estimate of endotoxin being 1% of the dry weight, by this method an inoculum of 10^{12} CFU has approximately 2.5 mg of endotoxin. By comparison, infusion of 4 ng of endotoxin per kg into a 70-kg man results in an infusion of 1/10,000 of the endotoxin load in 10^{12} CFU, or 280 ng. Further, when calculated on a weight basis, the infusion of 10^{12} CFU results in a dose of approximately 250,000 ng/kg for a 10-kg animal.

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