Pseudomonas aeruginosa Compared with Escherichia coli Produces Less Endotoxemia but More Cardiovascular Dysfunction and Mortality in a Canine Model of Septic Shock*

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We investigated the effects of two different Gram-negative bacteria and radiation-induced leukopenia on endotoxemia, cardiovascular abnormalities, and mortality in a canine model of septic shock. Serial hemodynamics were measured in conscious dogs using radionuclide heart scans and thermodilution cardiac output catheters. Plasma endotoxin concentrations were determined with a chromogenic Limulus amebocyte lysate assay. Viable Pseudomonas aeruginosa or Escherichia coli implanted intraperitoneally produced concordant hemodynamic patterns of septic shock (p < 0.01). Endotoxin concentrations were more than tenfold lower in dogs infected with P aeruginosa compared with E coli (p < 0.0001). Despite lower endotoxin levels, P aeruginosa–infected dogs had a higher mortality (p < 0.01), more severe hypotension (p < 0.05), and greater depression of the left ventricular ejection fraction (p < 0.05) than dogs with E coli sepsis. A nonlethal E coli challenge combined with leukopenia (induced by a nonlethal dose of radiation) resulted in a mortality of 60 percent (p < 0.01) without greater cardiovascular dysfunction or higher endotoxin concentrations. These findings suggest that bacterial products other than endotoxin and host-related factors may be important contributors to the toxicity, cardiovascular instability, and mortality of Gram-negative septic shock. Quantitative determinations of plasma endotoxin are unlikely to correlate with the clinical severity of septicemia in heterogeneous patient populations infected with different Gram-negative organisms.

Bacteremia caused by Gram-negative facultative or aerobic rods is a major source of morbidity and mortality among hospitalized patients.1-8 Endotoxin, a lipopolysaccharide (LPS) found in Gram-negative bacteria, is considered to be the principal toxin responsible for the development of septic shock in these infections.4,5 Treatment strategies such as "cross-reactive" anti-core LPS antibodies have been based on this hypothesis.9,10 The detection of endotoxin in humans, however, has correlated inconsistently with important clinical events,11-13 even though the Limulus amebocyte lysate (LAL) assay is sensitive,14 relatively specific,13,15 and correlates with in vivo toxicity.16 Potential reasons for this discrepancy include variability between lyseate preparations,12 circulating inhibitors,17,18 and rapid clearance of endotoxin from the circulation.19,20 However, a fully satisfactory explanation remains to be established.

Recent clinical studies have demonstrated that organisms devoid of endotoxin (Gram-positive bacteria and fungi) are capable of producing a pattern of cardiovascular injury indistinguishable from that caused by Gram-negative sepsis.21-25 In a canine model of septic shock, we found that the hemodynamic changes produced by Staphylococcus aureus and Escherichia coli identical, yet endotoxemia did not occur in septic shock induced by S aureus.24 These findings suggest that bacterial products other than endotoxin can activate a common pathway that results in similar

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The opinions and assertions contained herein are the private ones of the authors, and are not to be construed as official or reflecting the views of the Armed Forces. The experiments reported herein were conducted according to the principles set forth in "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council, Department of Health, Education and Welfare publication no. 74-23 (National Institutes of Health).


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1480 Canine Septic Shock (Danner et al)
CANINE MODEL OF SEPTIC SHOCK

Various types and doses of infecting agents implanted into the peritoneum

Baseline
Surgery
Peritonitis
Recovery
DAYS

-7
0
+1
+2
+3
+7
+10

Comprehensive Evaluation (CE)*
Radionuclide Cineangiography (RNCA)

*Comprehensive Evaluation
a. Conventional hemodynamics using femoral and pulmonary artery catheters are done simultaneous with an RNCA
b. Laboratory analysis
c. Volume infusion 80 ml/kg over 1 hour
d. Repeat (a) after volume infusion

FIGURE 1. This figure outlines the design and time course of the experiment. All dogs in the study had a clot implanted into the peritoneal cavity. The clot was either sterile (controls) or infected with a known quantity of either P aeruginosa or E coli.

cardiovascular injury and mortality. If some Gram-negative bacteria produce non-LPS toxins capable of triggering this common pathway, then anti-endotoxin therapies might not be universally efficacious in all cases of Gram-negative septic shock.

The clinical significance of plasma endotoxin concentrations during septic shock may also depend on host factors. Some patients may be more sensitive to the release of harmful endogenous mediators by endotoxin,25 and in others the presence of anti-LPS antibodies may be protective.26 An endotoxin challenge that is well tolerated by a healthy adult might result in shock and death in a host already compromised by injury or illness. Overall, the effects of underlying disease, immune suppression, and other variables on the consequences of endotoxia are poorly understood.

In this investigation using a canine model of septic shock,27,28 we compared the endotoxia, cardiovascular abnormalities, and mortality produced by two different Gram-negative bacteria that frequently cause septic shock in patients, E coli and Pseudomonas aeruginosa. Further, we evaluated the effect of leukopenia, a host-related risk factor for infection, on the cardiovascular abnormalities and endotoxia of Gram-negative septic shock.

METHODS

Experimental Design

This study employed a well-characterized canine model of human septic shock.27,28 The protocol (Fig 1) has been described previously.27,28 Comprehensive evaluations were performed at baseline (seven days prior to the onset of sepsis), and repeated on days 1, 2, and 10 after infection. These evaluations included conventional hemodynamics using femoral and pulmonary artery catheters, simultaneous radionuclide gated blood pool scans, and laboratory blood tests, including blood cultures. Cardiac output studies were done in awake animals before and after fluid loading (80 ml/kg) at each time point. Catheters, which were inserted percutaneously using only local anesthesia (lidocaine 1 percent), were removed at the end of each study day. Descriptions of catheter placement, physiologic measurements, and routine laboratory profiles are reported elsewhere.27,28

A total of 72 dogs were used in the current investigation. Table 1 outlines the groups studied by type and dose of bacteria and the dose of radiation. Data from 30 of the dogs used in these experiments have been reported previously.24,27,28 Infections were established as previously described.27 On day 0, fibrin clots containing known doses of bacteria in colony forming units per kilogram (CFU/kg) were surgically implanted into the peritoneal cavity of two-year-old purpose-bred beagles (10 to 14 kg) via a laparotomy under general anesthesia. This resulted in bacteremia for three to four days. Cardiovascular evaluations were not obtained until at least 24 hours after surgery to allow full recovery from anesthesia.

Ten dogs were exposed to a nonlethal dose of cobalt 60 radiation, which was known to result in transient leukopenia five to ten days

<table>
<thead>
<tr>
<th>Table 1—Experimental Groups by Type and Dose of Bacteria, Dose of Radiation, and Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacteria</strong></td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>1. Sterile clot</td>
</tr>
<tr>
<td>2. E coli</td>
</tr>
<tr>
<td>3. E coli</td>
</tr>
<tr>
<td>4. E coli</td>
</tr>
<tr>
<td>5. P aeruginosa</td>
</tr>
</tbody>
</table>

*Colonies forming units × 10⁶ per kilogram.
†Dogs previously reported.24,27,28
Figure 2. Each graph plots the time course (solid line) of a single hemodynamic parameter (MAP, CI, or SVI) for different study groups designated by the type of clot that was implanted. For each graph, the horizontal shaded area is the mean ± SEM for 100 normal dogs, which is shown for comparison. The dashed line originating from the solid line is the response to volume infusion at each time point.

later, to determine the effect of leukopenia on endotoxemia during septic shock. Unanesthetized animals were secured in plexiglass holders and bilaterally exposed to a midline tissue dose of 200 centiGrays (cGy) of cobalt-60 radiation at 10 cGy per minute. Depth-dose measurements were made at the center of a cylindrical phantom (diameter of 15.2 cm, composed of 0.32-cm lucite filled with muscle-equivalent liquid). Actual irradiations were monitored with ionization chambers. Six days after radiation injury at the time of maximal leukopenia, animals were implanted with E. coli-infected clots and studied as described above.

Bacteriology and Fibrin Clot Preparation

The strain of E. coli 06:H8 used in these studies was isolated from a patient with urinary tract sepsis. The P. aeruginosa was obtained from a patient with a fatal case of septic shock. Bacteria were stored in 1-ml aliquots of bactopeptone broth (Difco, Detroit, MI) and glycerol at −70°C. Bacterial doses and fibrin clots were prepared as previously described.7.14 The doses of bacteria were quantified turbidimetrically using a standard curve based on actual viability counts. Bacteria for direct LAL testing were prepared in the same manner.

Testing of the P. aeruginosa Isolate for Exotoxin A Production by Western Blotting

Special growth media was prepared to maximize exotoxin A yields.23-25 Trypticase soy broth (Baltimore Biological Laboratory, Cockeysville, MD), 38 g/L, was iron depleted by adding the chelating agent, Chelex 100 (Bio-Rad, Rockville, Centre, NY), 10 g/L, followed by stirring for 12 h at 5°C. The broth was then filtered through Whatman No. 2 filter paper before undergoing ultrafiltration using a 10,000-MW cutoff membrane (AMICON, Danver, MA). The media was supplemented with glycerol 1 percent and monosodium glutamate 0.05 mol/L. Nitrilotriacetic acid (Sigma Chemical Co., St. Louis, MO) was added (1.9 g/L), the pH was adjusted to 7.0, and the broth was sterilized.

Our experimental strain of P. aeruginosa and strain PA-103 were inoculated into separate aliquots of the special media. PA-103 is a known exotoxin A producer and was used as a positive control to validate our methods. After overnight growth, both cultures were centrifuged and the supernatants were decanted. The supernatants were diluted 1:10 (V:V) with “stacking gel” buffer containing 125 mmol/L of Tris-HCl, at a pH of 8.8, and then concentrated 50-fold by ultrafiltration using 10,000-MW cutoff membranes (Amicon, Danvers, MA).

The concentrates and a purified recombinant exotoxin A standard (gift of Dr. David J. FitzGerald, Laboratory of Molecular Biology, National Cancer Institute) were electrophoresed on a 10 percent polyacrylamide gel followed by overnight electrophoretic blotting (Bio-Rad, Rockville Centre, NY) onto nitrocellulose membranes (Schleicher and Schuell, Inc, Keene, NH) using a pH of 8.3, 250 mmol/L of Tris, 192 mmol/L of glycine, and 20 percent methanol transfer buffer.16 Blots were incubated with rabbit anti-exotoxin A hyperimmune serum (gift of Dr. David J. FitzGerald) at a 1:250 dilution, followed by goat anti-rabbit antibody conjugated to horseradish peroxidase (1:1,000 dilution), and finally developing solution (Bio-Rad, Rockville Centre, NY). This method is able to detect 10 ng/ml of exotoxin A in broth supernatant.

Endotoxin Determinations

A quantitative chromogenic LAL assay (Whittaker M.A. Bioproducts, Walkersville, MD), sensitive to 10 pg/ml of US standard

<table>
<thead>
<tr>
<th>Type of Clot</th>
<th>Mean Arterial Pressure (mmHg)</th>
<th>Cardiac Index (ml/kg/min)</th>
<th>Stroke Volume Index (ml/kg)</th>
<th>Pulmonary Capillary Wedge Pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterile clot (controls)</td>
<td><img src="data/journals/chest/21622/1482_g2a.png" alt="Graph" /></td>
<td><img src="data/journals/chest/21622/1482_g2b.png" alt="Graph" /></td>
<td><img src="data/journals/chest/21622/1482_g2c.png" alt="Graph" /></td>
<td><img src="data/journals/chest/21622/1482_g2d.png" alt="Graph" /></td>
</tr>
<tr>
<td>P. aeruginosa 14 × 10⁶</td>
<td><img src="data/journals/chest/21622/1482_g2e.png" alt="Graph" /></td>
<td><img src="data/journals/chest/21622/1482_g2f.png" alt="Graph" /></td>
<td><img src="data/journals/chest/21622/1482_g2g.png" alt="Graph" /></td>
<td><img src="data/journals/chest/21622/1482_g2h.png" alt="Graph" /></td>
</tr>
<tr>
<td>E. coli 14 × 10⁶</td>
<td><img src="data/journals/chest/21622/1482_g2i.png" alt="Graph" /></td>
<td><img src="data/journals/chest/21622/1482_g2j.png" alt="Graph" /></td>
<td><img src="data/journals/chest/21622/1482_g2k.png" alt="Graph" /></td>
<td><img src="data/journals/chest/21622/1482_g2l.png" alt="Graph" /></td>
</tr>
<tr>
<td>Radiation and E. coli 14 × 10⁶</td>
<td><img src="data/journals/chest/21622/1482_g2m.png" alt="Graph" /></td>
<td><img src="data/journals/chest/21622/1482_g2n.png" alt="Graph" /></td>
<td><img src="data/journals/chest/21622/1482_g2o.png" alt="Graph" /></td>
<td><img src="data/journals/chest/21622/1482_g2p.png" alt="Graph" /></td>
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</tbody>
</table>

Days Pre or Post Implantation of Infected or Sterile Clot

† These animals were made neutropenic with 200 cGy of cobalt-60 radiation prior to clot implantation.
endotoxin, was used to determine endotoxin concentrations as previously described.\textsuperscript{24} Serial blood samples (3 ml each) were collected in sterile, pyrogen-free glass tubes containing heparin (2 USP units/ml of blood) at baseline and days 1, 2, and 10 after surgery. These time points were chosen to coincide with maximal depression and full recovery of myocardial dysfunction as determined from previous studies.\textsuperscript{25,26,27} The \textit{P. aeruginosa}-infected dogs and four of the \textit{E. coli}-infected dogs (14 x 10\textsuperscript{8} CFU/kg) also had endotoxin determinations at 6 and 12 hours after clot implantation. These data were collected to determine that early peaks or surges in endotoxemia were not missed by the chosen time points. The endotoxin contents of the \textit{E. coli} and \textit{P. aeruginosa} strains used in this study were determined on freshly grown and washed bacteria using serial dilutions.

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\textbf{Table 2—Endotoxemia during Septic Shock: Median Values and Ranges}

<table>
<thead>
<tr>
<th>Study Group</th>
<th>Median Endotoxin Concentrations*</th>
<th>Range of Interquartile Endotoxin Concentrations</th>
<th>No. of Dogs Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Sterile clot\textsuperscript{†}</td>
<td>0.5</td>
<td>0.2-0.9</td>
<td>6</td>
</tr>
<tr>
<td>2. \textit{E. coli}\textsuperscript{‡}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>\textit{7 x 10\textsuperscript{8}}</td>
<td>58.9</td>
<td>16.9-123.6</td>
<td>6</td>
</tr>
<tr>
<td>\textit{14 x 10\textsuperscript{8}}</td>
<td>35.0</td>
<td>20.1-99.8</td>
<td>10</td>
</tr>
<tr>
<td>\textit{14 x 10\textsuperscript{8}} (with radiation)</td>
<td>32.7</td>
<td>6.3-84.7</td>
<td>10</td>
</tr>
<tr>
<td>3. \textit{P. aeruginosa}\textsuperscript{§}</td>
<td>2.3</td>
<td>1.1-5.0</td>
<td>28</td>
</tr>
</tbody>
</table>

*Results are reported as endotoxin units (EU) per milliliter of plasma. One EU = the activity of 100 pg of US standard endotoxin.

\textsuperscript{†}Sterile clot dogs had endotoxin levels significantly lower than any of the infected groups (p<0.0005).

\textsuperscript{‡}\textit{E. coli}-infected groups did not differ significantly in endotoxin levels (p>0.3).

\textsuperscript{§}\textit{P. aeruginosa}-infected dogs developed significantly lower levels of endotoxemia than dogs infected with \textit{E. coli} (p<0.0001).
Median endotoxin concentrations on days 1 and 2 were compared nonparametrically (due to variability in E. coli groups) with a Mann-Whitney U test. Significance was adjusted with a modified Bonferroni. 

Mean decreases in mean arterial pressure (MAP) and left ventricular ejection fraction (LVEF) were compared among groups using unpaired, two-tailed, t tests. These results were declared significant only if the resulting analysis of variance (ANOVA) was significant, the so-called Protected Significant Difference Test. Other comparisons of mean values were performed with the appropriate t test, as indicated. Relative frequencies of death among groups were compared using the Fisher exact test.

**RESULTS**

**Clinical Manifestations, Blood Cultures, and Laboratory Tests**

As previously reported, the intraperitoneal implantation of infected clot produced fever and prostration in all animals. Blood cultures grew the organism placed in the clot on days 1, 2, and 3 after surgery. Thereafter, blood culture positivity began to decrease. In survivors, blood cultures were sterile by seven days after the onset of infection. Control dogs with sterile clots were afebrile, had sterile blood cultures, and were healthy throughout the study.

Creatinine, urea, pH, and PO2 remained within the normal range for all study groups. Hemoglobin, sodium, potassium, bicarbonate, chloride, glucose, and calcium levels were similar in all infected dogs. The 200 cGy (total body dose) of cobalt 60-generated gamma radiation, given six days prior to E. coli implantation (14 × 10^8 CFU/kg), resulted in marked leukopenia during sepsis. The day 1 peripheral white blood cell counts in E. coli-infected dogs were 800 ± 143 and 16,200 ± 1,444/cu mm (mean ± SEM) in the irradiated and nonirradiated dogs, respectively (p<0.001). In 20 non-infected beagles, this dose of radiation produced a transient fall in the peripheral white blood cell count to 23 percent ± 5 percent of baseline values (p<0.005) over six to ten days, but no other manifestations of radiation injury.

**Comparison of Qualitative Hemodynamic Changes and Mortality**

Serial changes in cardiovascular performance were observed during the course of the experiment in all infected dogs (Fig 2 and 3, data not shown for E. coli 7 × 10^8 CFU/kg). Animals implanted with sterile clots, however, maintained their hemodynamic parameters within the normal range (Fig 2 and 3) throughout the study period (baseline, and days 1, 2, and 10 after surgery). To determine if the pattern of hemodynamic changes was similar for different bacteria, the Kendall coefficient of concordance (see "Methods") was calculated for each parameter. The coefficient of concordance determines if graded hemodynamic responses occurred at similar time points in different groups.

All infected dog groups (Table 1) were used in the comparison. A strong concordance was found for each of the serial hemodynamic parameters followed during sepsis: LVEF = 0.93 (p<0.01); MAP = 0.93 (p<0.01); cardiac index (CI) = 0.78 (p<0.03); stroke volume index (SVI) = 0.85 (p<0.02); end-systolic volume index (ESVI) = 0.78 (p<0.03); and end-diastolic volume index (EDVI) = 0.68 (p<0.05). On day 2 of sepsis, after volume infusion (denoted by asterisks in Fig 2 and 3), all groups of infected dogs (including P. aeruginosa), compared with baseline values, demonstrated a decreased LVEF (p<0.001), increased EDVI (p<0.05), and increased CI (p<0.01). This cardiovascular profile is similar to the hyperdynamic response observed in human septic shock and has been previously described in this canine model for E. coli and S. aureus septic shock.

Overall mortality differed markedly among the study groups (Table 1). All dogs receiving E. coli at doses of 7 and 14 × 10^8 CFU/kg survived. *Pseudomonas aeruginosa* at a dose that was nonlethal for E. coli (14 × 10^8 CFU/kg) resulted in a significant mortality, 11 of 32 animals (p<0.01; Fisher exact test). Total body ionizing radiation of 200 cGy increased mortality from 0 to 60 percent (p<0.01; Fisher exact test) in animals infected with E. coli (14 × 10^8 CFU/kg).

**Plasma Endotoxin Concentrations and Quantitative Hemodynamic Changes**

Endotoxin concentrations determined during septic shock for each of the study groups are shown in Table 2. As previously reported, dogs implanted with sterile clot did not have development of significant endotoxemia compared with baseline values. Dogs infected with E. coli and *P. aeruginosa* had detectable endotoxemia on day 1 after surgery that decreased but remained significantly elevated (p<0.01) at 48 hours. In the four E. coli dogs tested at earlier time points, plasma endotoxin concentrations at 6 and 12 hours after clot implantation were similar (p = NS) to day 1 values. *Pseudomonas aeruginosa* infection resulted in very low or undetectable concentrations of plasma endotoxin at these early time points.

**Table 3—Mean Decreases in Mean Arterial Pressure and Left Ventricular Ejection Fraction**

<table>
<thead>
<tr>
<th>Study Group</th>
<th>Decrease in MAP, mm Hg</th>
<th>Decrease in LVEF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. E. coli</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 × 10^6</td>
<td>−24 ± 6</td>
<td>−0.16 ± 0.03</td>
</tr>
<tr>
<td>14 × 10^6</td>
<td>−30 ± 5</td>
<td>−0.27 ± 0.03</td>
</tr>
<tr>
<td>14 × 10^6 (with radiation)</td>
<td>−32 ± 4</td>
<td>−0.35 ± 0.02</td>
</tr>
<tr>
<td>2. P. aeruginosa</td>
<td>−41 ± 3</td>
<td>−0.24 ± 0.02</td>
</tr>
</tbody>
</table>

*Results expressed as mean decreases ± SEM.

†P. aeruginosa (14 × 10^8 CFU/kg)-infected dogs had greater falls in MAP and LVEF than low dose E. coli (7 × 10^8 CFU/kg)-infected dogs (p<0.05).
Pseudomonas aeruginosa-infected dogs had markedly lower endotoxin levels compared with the E. coli groups (p<0.0001). Eleven of 32 animals died in the P. aeruginosa group compared with none of the nonirradiated E. coli dogs (7 and 14 × 10⁶ CFU/kg), despite circulating endotoxin concentrations that were more than tenfold lower in the P. aeruginosa-infected dogs (Table 2). Other parameters (Table 3) also pointed to a pattern of greater severity of illness in the setting of relatively low levels of endotoxemia in P. aeruginosa vs E. coli groups. As shown, P. aeruginosa (14 × 10⁶ CFU/kg) produced greater falls in MAP and LVEF (p<0.05) than E. coli (7 × 10⁶ CFU/kg), despite the higher endotoxin concentrations produced by the latter.

This disparity between endotoxin concentrations and clinical end points in the P. aeruginosa group could not be explained by additive toxicity from exotoxin A, a potent toxin thought to be important in Pseudomonas infections. The P. aeruginosa strain used in these experiments released no detectable exotoxin A as measured by Western blot after overnight growth in high-yield media (Fig 4). Further, the endotoxin content per bacterium, as determined by LAL assay, was similar for the E. coli and P. aeruginosa used in this study, 25.5 fg/CFU and 26.6 fg/CFU, respectively (in equivalent weight of US standard endotoxin).

As stated above, radiation-induced leukopenia increased mortality from 0 to 60 percent in E. coli-infected dogs, but did not affect the pattern or severity of the hemodynamic abnormalities (Fig 2 and 3, and Table 3). The increased mortality seen in irradiated, E. coli-infected dogs was not caused by higher concentrations of circulating endotoxin (Table 2), despite the marked immune suppression.

DISCUSSION

The detection of endotoxemia with the LAL assay in human infection has in some studies correlated poorly with clinical events and outcomes.11,12 These investigations included patients from diverse clinical settings13 who were infected with a wide variety of Gram-negative bacteria36,37 that differed in virulence factors, growth characteristics, and endotoxin structure. In this study, using a canine model of septic shock, two different Gram-negative bacteria, E. coli and P. aeruginosa, were found to produce qualitatively identical patterns of hemodynamic change. The particular strain of P. aeruginosa used in these experiments, however, resulted in tenfold lower plasma endotoxin concentrations, but greater cardiovascular abnormalities and a higher mortality compared with the E. coli isolate. This dissociation between endotoxemia and the severity of sepsis argues that in some Gram-negative infections, bacterial products other than endotoxin may be important contributors to cardiovascular injury and mortality in septic shock.

If correct, therapeutic strategies aimed at inhibiting or blocking endotoxin, such as anti-core LPS antibodies9,10 and lipid X,39 may not be universally efficacious in all cases of Gram-negative septic shock.

Extrapolating these findings to human studies on endotoxemia suggests that plasma endotoxin concentrations are unlikely to correlate with the severity or course of infection when comparisons are made among different genera of gram-negative bacteria. Most previous studies of endotoxemia in human sepsis relied on LAL assays based on gelation that were only sensitive to 1 ng/ml or more of endotoxin.18 In the current experiment, P. aeruginosa produced such low levels of endotoxemia that dogs dying of sepsis from this Gram-negative organism would have been LAL assay negative by the gelation method.

The potency of bacterial endotoxins in the Limulus assay is dependent on both the source of the endotoxin and the method of preparation.16 The findings of this
study could be explained if the LPS produced by \( P \) aeruginosa were very toxic in vivo but had only weak activity in the LAL assay. The in vivo activity of an endotoxin, however, has been shown to correlate with its potency in the LAL assay.\(^{18}\) Comparison of \( E \) coli and \( P \) aeruginosa endotoxins by Limulus assay have found them to be very similar in activity.\(^{29}\) Further, the strain of \( E \) coli and \( P \) aeruginosa used in this study had similar amounts of endotoxin per bacterium by our LAL assay. Lastly, it has been suggested that \( P \) aeruginosa LPS may be less, not more, toxic than the LPS produced by other Gram-negative bacteria.\(^{40}\) Recent studies, however, have found that the endotoxins of \( E \) coli and \( P \) aeruginosa are roughly equipotent in vivo.\(^{41}\)

In a previous study using this canine model, we demonstrated that \( S \) aureus produced a cardiovascular pattern of septic shock identical to \( E \) coli, but in the absence of any detectable endotoxin.\(^{24}\) This indicates that bacterial products other than endotoxin can activate a common pathway resulting in similar cardiovascular injury and mortality. The current investigation suggests that endotoxin may not be the universal, or even the most important, toxin in all infections caused by Gram-negative bacteria. These findings, taken together, support the hypothesis that a number of bacterial products can activate an endogenous mediator (such as tumor necrosis factor)\(^{42}\) or cascade that acts as the final common pathway to hemodynamic instability and death in septic shock.\(^{43}\)

\( P \)seudomomas aeruginosa\ is known to produce a number of proteases and exotoxins that may be related to its pathogenicity.\(^{41}\) Of these, exotoxin A, a potent inhibitor of protein synthesis, has been most closely linked to systemic toxicity and death.\(^{44}\) Exotoxin A is produced by about 90 percent of clinical \( P \) aeruginosa isolates,\(^{45}\) and naturally acquired antibodies to exotoxin A correlate with survival from \( P \) aeruginosa sepsis in humans.\(^{46-47}\) Our finding of greater toxicity but lower endotoxin concentrations associated with \( P \) aeruginosa compared with \( E \) coli might be explained by the additive or synergistic action of exotoxin A. The \( P \) aeruginosa isolate used in these experiments, however, did not produce exotoxin A. This argues that additional, less well-defined, bacterial products may significantly contribute to the pathogenesis of the septic shock syndrome.

As expected, radiation-induced leukopenia dramatically increased the mortality of septic shock. Uncontrolled infection due to immune suppression might have been responsible for this high mortality. Interestingly, this was not reflected by greater or more prolonged endotoxemia. This suggests that, in the setting of immune suppression (in this case radiation-induced leukopenia), levels of endotoxin that would otherwise be nonlethal may result in death. The concept of combined injury is not new, but these findings underscore the potential for complex interactions between the infecting organisms, endotoxin concentrations, and host factors that may affect outcome.

Multiple investigators support the hypothesis that release of endotoxin from Gram-negative bacteria is the principal pathogenic mechanism in septic shock.\(^{1-7,9,10,13,14,48}\) Previously we have shown that Gram-positive bacteria, in the absence of detectable endotoxin, can produce lethality and all of the cardiovascular changes of septic shock.\(^{24}\) In this study, two types of Gram-negative bacteria produced the same cardiovascular changes of septic shock, but with a negative correlation between the development of endotoxemia and clinical outcomes. It is unlikely that the development of endotoxemia in \( E \) coli sepsis was protective, suggesting that agents other than endotoxin were important pathogenic factors in \( P \)seudomonas-infected dogs. The endotoxin concentrations in this study cannot be generalized to all strains of \( E \) coli and \( P \) aeruginosa. However, the previous data from studies of Gram-positive infection\(^{24}\) combined with data from this study strongly suggest that endotoxin is not the universal mediator of septic shock.

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