Mortality and Bacteriology of Sepsis following Cecal Ligation and Puncture in Aged Mice

SCOTT R. HYDE,1 REX D. STITH,2 AND RODERICK E. McCALLUM1*

Department of Microbiology and Immunology,1 and Department of Physiology and Biophysics,2 University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma 73190

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Epidemiologic data suggest that elderly adults are more susceptible to invasive bacterial infection by indigenous gut flora than are younger adults. The purpose of this investigation was to characterize a murine model of clinically encountered peritonitis in the aged. We subjected three different age groups (young, 16 weeks; mature, 12 months; senescent, 24 months) of C57BL/6Nia mice to surgically induced peritonitis by the cecal ligation and puncture procedure. Senescent mice died in a significantly shorter time following surgery than mature mice (median time to death, 24.4 versus 38.5 h, respectively; P ≤ 0.001). Blood, liver, spleen and occasionally, ceca were obtained at 2 and 12 h after the cecal ligation and puncture procedure and immediately following death. To characterize the bacterial kinetics of the model, qualitative and quantitative aerobic, anaerobic, and coliform cultures were performed. No age-related differences were found in the types of bacteria isolated throughout the time course of progressive sepsis. In mice in the mature and senescent age groups, at 2 and 12 h postsurgery, gram-negative anaerobes and gram-positive aerobes predominated in all tissues that were cultured. At the time of death, however, blood and tissue isolates consisted predominately of coliform bacteria. The shift from mixed infection during sepsis to predominantly gram-negative bacterial infection reflected a similar progressive shift in bacterial types found in the cecum. At death, senescent mice had 100-fold fewer coliform bacteria in the bloodstream than those found in mature mice (2.5 × 106 versus 4.6 × 109, respectively). The increased sensitivity of aged mice to invasive bacterial infection documented in this series of experiments accords well with human epidemiologic experience and demonstrates the appropriateness of the model for continued investigations of sepsis in the aged.

The gastrointestinal tract is a commonly reported source of bacteremia, which has been associated with fatality rates that exceed 30% (13). Advanced age has been identified as a major risk factor for the development of postoperative septic complications following abdominal surgery (14). Likewise, mortality rates from complicated appendicitis are reported to be 5- to 10-fold greater in elderly than they are in younger individuals (8). These epidemiologic data suggest that associated with the process of aging is an increased incidence and sensitivity to invasive bacterial infection with indigenous gut flora.

To develop an animal model of sepsis in the aged, we performed preliminary studies of lethality caused by surgically induced peritonitis in mice of different ages. The cecal ligation and puncture procedure (CLP) was selected because it mimics clinically encountered peritonitis in the following ways. Indigenous gut flora are seeded into the systemic circulation from a remote septic focus, blood cultures are positive, and the insult is polymicrobial (20). Also, the CLP model has been well characterized and has been used to investigate the protective effects of endotoxin, interleukin-1, and tumor necrosis factor pretreatment in lethality studies and to characterize the bacteria found in the blood and peritoneal fluid of septic mice (9, 17, 18). As observed in humans with sepsis, mice exposed to endotoxin or subjected to CLP-induced peritonitis manifest an early, hyperdynamic metabolic response followed by a late, hypodynamic phase and death (16, 17). The shift from hyperdynamic to hypodynamic status following challenge with endotoxin or CLP is consistently reproducible and predictable throughout the time course of progressive sepsis (17).

Age-related differences in plasma corticosterone and glucose homeostasis following exposure to endotoxin have been documented in aging mice (16), although a comprehensive study of experimental sepsis caused by indigenous flora in aging mice has not yet been reported. This study was undertaken, therefore, to characterize the bacterial kinetics of the CLP model at several pathophysiologic stages of sepsis and to compare the sensitivities of aged mice to a specific bacterial insult. An increased sensitivity of senescent mice to sepsis following CLP was manifested by shorter survival times following surgery and lower bacterial counts in blood and tissue at the time of death. The results provide quantitative support for the hypothesis that aged mammals are more sensitive than younger mammals to the lethal effects of invasive bacterial infection with indigenous gut flora.

MATERIALS AND METHODS

Animals. Male C57BL/6Nia mice of three different ages were obtained from the National Institute of Aging (Bethesda, Md.) through Charles River Breeding Laboratories, Inc. (Wilmington, Mass.). The groups consisted of young mice (age, 16 weeks), mature mice (age, 12 months), and senescent mice (age, 24 months). The average life span of male virgin C57BL/6Nia mice is 27.2 ± 1.1 months (1). Mice were maintained at 23°C with access to food and tap water ad libitum. The animals were acclimated to the laboratory environment for at least 1 week prior to surgical manipulation.

Surgical procedure. Peritonitis was surgically induced under light ether anesthesia by aseptic techniques. To minimize trauma, a 1-cm incision was made into the left upper quadrant of the peritoneal cavity (normal location of the...
The cecum was exposed, and the intestinal contents were milked from the large bowel into the cecum until it became mildly distended. To maintain bowel continuity, a tight ligature was placed around the cecum with 4-0 sutures, distal to the insertion of the small bowel. Two puncture wounds were made into the antimesenteric serosal surface of the cecum with a 20-gauge needle, and a small amount of cecal contents was expressed through the wounds. The cecum was replaced into the peritoneal cavity. The anterior peritoneal wall was closed with 4-0 silk sutures, and the skin was apposed with surgical staples. The incision was dressed with a topical antibiotic (0.2% nitrofurazone; Vedco, Overland Park, Kans.), and the animals were allowed to recover.

In lethality studies, 1 ml of sterile saline was injected subcutaneously to prevent postoperative hypotension. Since the average body weight was 35.7 g for mature mice and 30.2 g for senescent mice, the fluid resuscitation volume was greater in the older animals. However, in subsequent bacteriology studies in which no fluid was administered following surgery, no significant difference (P = 0.80) in mean time to death, regardless of age, was seen to be due to fluid resuscitation. Mice were physically examined and were not admitted to the experimental protocol if signs of fever, malaise, immobility, upper respiratory disease, or tumors were found. Aging animals with degenerative lesions of the seminal glands were not excluded from the study.

Progressive pathophysiologic metabolic response following CLP. Five physiologic parameters were measured to facilitate characterization of the progression of sepsis from hypermetabolism to hypometabolism following CLP. Six mice were tested prior to surgical manipulation, and six mice were tested at 2 and 12 h post-CLP. Systolic blood pressure, heart rate, body temperature, blood glucose levels, and liver glycogen levels were measured. The hypermetabolic phase of sepsis was evidenced by hyperglycemia, tachycardia, and hypertension, while hypometabolism was characterized by a reversal of these parameters (21). Blood glucose was determined with a glucose analyzer (Beckman Instruments, Inc., Fullerton, Calif.) by the glucose oxidase method (4). Liver glycogen was purified by ethanol precipitation and was measured as glucose following α-amylodiglucosidase treatment (10). Rectal temperatures were recorded with a digital thermometer while the animals were held at room temperature. Heart rate and blood pressure measurements were obtained with a tail cuff pressure transducer and polygraph (Narco Biosystems, Houston, Tex.).

Lethality studies. Approximately 35 mice from each of the three age groups were used for lethality studies. Following CLP, animals were allowed to recover from anesthesia and were observed for 48 h. The time to death after surgery for each animal was noted and recorded.

Bacteriology of sepsis. A total of 18 mature mice and 18 senescent mice were made septic via CLP; and samples of blood, liver, and spleen were submitted for bacteriologic investigation at intervals after surgery. Samples from 6 of the 18 mice from each age group were taken when the mice were in the hypermetabolic phase of sepsis (2 h post-CLP); samples were also taken from 6 mice in the hypometabolic phase (12 h post-CLP) and from 6 mice at the time of death. Following the sampling at 12 h, the remaining mice were observed at approximately 30-min intervals for signs of pending death (moribund, labored breathing, shaking, and hypothermia), and blood and tissue samples were obtained immediately at the time of death. There was no delay in obtaining postmortem samples for bacteriologic culture. Quantitative and qualitative bacteriology was performed on each tissue sample. Samples from the cecum for culture were taken from several animals at 2 and 12 h post-CLP and at the time of death.

Bacterial culture methods. Blood was obtained under ether anesthesia or at the time of death from the brachial artery and immediately serially diluted 10-fold in pre-reduced, anaerobically sterilized salts solution (6). The diluted blood was transferred rapidly to pre-reduced brain heart infusion (BHI) agar plates, supplemented with 0.02% vitamin K–1.0% hemin–0.05% cysteine (6), and placed immediately into an anaerobic glove box. The remaining blood was plated in duplicate onto MacConkey and BHI agar, which were incubated aerobically for 48 h at 37°C.

Tissue samples were weighed and rinsed two times in 4.5 ml of pre-reduced, anaerobically sterilized salts solution. Nine volumes of pre-reduced, anaerobically sterilized salts solution were added to the tissue, and the mixture was homogenized with a Tri-R tissue homogenizer. Serial 10-fold dilutions of the resultant tissue suspension were prepared, and cultures were processed similarly to blood cultures. The inoculated plates were placed immediately in the anaerobic chamber; plates were incubated for 48 h prior to colony counting, to facilitate delineation of colony morphology. Plates that contained between 30 and 300 colonies were counted, and if 4 or more different colony types were present, only the 3 most numerous colony types were selected for identification. Anaerobic bacterial isolates were identified by gas-liquid chromatography and with biochemical test strips (API-20A; Analytab Products, Inc., Houston, Tex.). Gram-negative aerobes were identified with API-20E test strips, and gram-positive aerobes were identified by the methods outlined in Fig. 1.

Statistics. Mortality data were analyzed by the method of Knapp and Wise (7), which was specifically developed to deal with data consisting of survival times and with the presence of live animals at the end of the observation period. Bacteriologic data were analyzed by Student's t test and chi-square analysis (Statgraphics; STSC, Inc. Rockville, Md.).

RESULTS

Lethality studies. Figure 2A was constructed from the mortality data collected for mice in each of the three age groups and depicts the percent cumulative lethality at various times after surgery. The Gehan generalized Wilcoxon test (22) was used to compare the survival times of mice in the different age groups. Such analyses revealed that the survival time was not significantly greater for mature mice compared with that for young mice. Likewise, the survival time of young mice was not statistically greater than that of senescent mice. However, the survival time of mature mice was significantly longer than that of senescent mice (P ≤ 0.001).

Percent cumulative lethality. Since percent cumulative lethality data are difficult to compare quantitatively, the sigmoidal curves in Fig. 2A were converted to linear plots by logit transformation (11), resulting in the parallel lines of Fig. 2B. When the logit was 0, the corresponding values of x represent the median time to death (MTD). This value was calculated for each group of mice and assessed statistically by the method of Newman-Keuls (22). The MTD was 24.4 h for senescent mice, 30.6 h for young mice, and 38.5 h for mature mice. The MTD for young mice was not statistically different from that of mice in either the mature or the senescent age group; however, the difference between the
FIG. 1. Flow chart of diagnostic microbiologic methods. Abbreviations: PRAS, prereduced, anaerobically sterilized, GPR, gram-positive rods; GNR, gram-negative rods; GPC, gram-positive cocci; BEA, bile esculin agar.

MTD for senescent mice versus that for mature mice was statistically significant ($P < 0.05$).

Pathophysiologic metabolic responses following CLP. Data characterizing the metabolic dynamics of CLP are presented in Table 1. Blood pressure, heart rate, and blood glucose levels were elevated at 2 h post-CLP, while liver glycogen and rectal temperatures were depressed. These findings were indicative of the acute hyperdynamic metabolic phase of sepsis. By 12 h post-CLP, all physiologic parameters measured were depressed, indicating the hypodynamic phase of sepsis.

Shift from predominately gram-positive to gram-negative bacteria as sepsis progressed. Because the lethality data revealed statistical differences only between mature and senescent mice, we elected to restrict bacteriologic studies to mice in these two age groups. In mice in both age groups, the species of bacteria isolated from the blood following CLP reflected those isolated from the liver and spleen. Likewise, the bacterial species isolated from the blood were similar between mice in the two age groups. The data in Table 2 represent the combined results of aerobic BHI blood cultures isolated at 2 and 12 h post-CLP and at the time of death from mice in both age groups. At 2 h post-CLP, there were six positive aerobic BHI blood cultures. All six cultures consisted of gram-positive bacteria. By 12 h post-CLP, there was a predominance of gram-positive organisms, with a single isolate of Escherichia coli. In blood cultures sampled at the time of death, however, gram-negative bacteria predominated (13 gram-negative isolates versus 5 gram-positive isolates). Chi-square analysis revealed a shift in predominating bacteria from gram positive to gram negative as sepsis progressed ($P \leq 0.002$).

Shift from predominately anaerobic to aerobic gram-negative bacteria as sepsis progressed. The data presented in Table 3 reveal combined results from anaerobic blood cultures of mice in both age groups. At 2 h post-CLP, anaerobic bacteria predominated. By 12 h, there was a mix of aerobes and anaerobes, and at the time of death aerobic coliform bacteria predominated. Chi-square analysis revealed a progressive shift in the type of bacteria isolated from the blood ($P = 3.9 \times 10^{-8}$).

Cecal cultures. In seven mice that were representative of both age groups, samples of the cecum were taken, in addition to those of blood, liver, and spleen, for culture. Ceca were sampled at 2 and 12 h post-CLP and at the time of death by culture methods identical to those described above for other tissues. Comparison was made between the three predominating colony types found on the aerobic BHI plates. If one of the three predominating isolates selected for identification from the cecum was also one of the three predominating isolates found in the other tissue samples, that cecal isolate received a concordance score of 1. If a predominating cecal isolate was not one of the three predominating species isolated from other tissues, that isolate received a discordance score of 1. At 2 h post-CLP, there were
15 concordant isolates and 4 discordant isolates. By 12 h there were 14 concordant versus 4 discordant isolates, and at the time of death there were 8 concordant isolates and 1 discordant isolate. In total, there were 36 concordant isolates and 9 discordant isolates. Chi-square goodness-of-fit analyses revealed a strong correlation between bacterial species that predominated in the cecum and those that predominated in other tissues (\( P = 0.00012 \)). In other words, the cecum was the primary focus of infection.

**Comparison of total versus coliform counts in blood, liver, and spleen.** The total bacterial counts (anaerobic BHI plates) versus total coliform counts (MacConkey agar plates) in blood, liver, and spleen are shown in Table 4. Total blood counts were derived by multiplying the bacterial count per milliliter of blood by the estimated total blood volume (75 ml/kg of body weight [12]). Total liver and spleen counts were derived by multiplying the bacterial count per gram of tissue by the weight of the organ. The values represent the mean for mice in each age group.

At 2 and 12 h post-CLP, total liver bacterial counts were consistently 2.5 log units greater than blood or spleen counts. These findings are consistent with the notion that the liver is the major organ for trapping and eliminating bacteria from the blood. At the time of death, however, liver counts were similar to blood and spleen counts.

As sepsis progressed in mice in both age groups, coliform counts increased in proportion to the total bacterial counts. At 2 h post-CLP, senescent animals lacked coliforms in blood, liver, and spleen, while approximately 0.1% of the liver bacterial load in mature animals was made up of coliform bacteria. By 12 h post-CLP, coliform counts accounted for approximately 1% of the total bacteria in the livers in mice in both age groups. Although liver bacterial counts in senescent mice appeared to be 10-fold higher than those in mature mice, the difference was not significant (\( P = 0.12 \)). At the time of death, total bacterial counts (anaerobic BHI plates) in mice in both age groups were approximately equal to the coliform counts (MacConkey’s agar), thus indicating a predominance of coliforms.

**Bimodal distribution of total blood counts for mature and senescent mice at the time of death.** Total bacterial blood counts (anaerobic BHI agar plates) for senescent mice versus those for mature mice sampled at the time of death are presented in Fig. 3A. The data are presented to highlight the variation in total counts within each age group and to emphasize the point that when death occurred, animals in both age groups were experiencing gram-negative sepsis (Fig. 3B). The mature mouse group had approximately 2 log units, or 100-fold, more bacteria in the blood at the time of death than those found in senescent mice. In mice in each age group, there were two subpopulations of animals. The differences between the subpopulations are presented in Table 5. Animals that died early following CLP had consistently low bacterial counts and mixed blood cultures, while those that lived longer tended to have high bacterial counts that consisted solely of coliform bacteria. The reason for the bimodal distribution of bacterial counts from septic animals was not apparent from gross postmortem examination.

Because there was a bimodal distribution of bacterial counts mice in each age group, comparison by parametric statistical analyses is not valid. However, we opine that 2 log units, or 100-fold, less bacterial load at the time of death suggests a biologic difference. By assuming that there is a biologic bimodal distribution of animals within each age group and by selecting the subpopulations of mice that died late with pure cultures and those that had high bacterial counts, parametric tests became appropriate since the subpopulations were normally distributed. Student’s \( t \) test analysis revealed a borderline statistical age-related difference in blood counts between mice in the two age groups (\( P = 0.053 \)).

**TABLE 2.** Shift in predominance of gram-positive to gram-negative bacteria in aerobic blood cultures obtained from C57BL/6NNia mice during sepsis following cecal ligation and puncture

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Total no. of isolates at the following times of culture post-CLPa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 h</td>
</tr>
<tr>
<td>Gram positive</td>
<td>6</td>
</tr>
<tr>
<td>Gram negative</td>
<td>0</td>
</tr>
</tbody>
</table>

\( a \) The following representative isolates were obtained at 2 h post-CLP: *Bacillus* spp. (4 isolates), *Corneybac terium* spp., and *Lactobacillus* spp. At 12 h post-CLP, the following representative isolates were obtained: *Lactobacil lus* spp. (3 isolates), group D streptococci, *Staphylococcus* spp., and *Escherichia coli*. At the time of death, the following representative isolates were obtained: *Escherichia coli* (8 isolates), *Enterobacter cloacae* (3 isolates), group D streptococci (3 isolates), *Pseudomonas fluorescens*, Protea mirabil ills, *Staphylococcus aureus*, and an aerobic gram-positive rod. \( P = 0.0023 \) by chi-square analysis.

**TABLE 3.** Shift in predominance of anaerobic to aerobic bacteria in blood cultures obtained from C57BL/6NNia mice during sepsis following cecal ligation and puncture

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Total no. of isolates at the following times of culture post-CLPa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 h</td>
</tr>
<tr>
<td>Anaerobes</td>
<td>18</td>
</tr>
<tr>
<td>Aerobes</td>
<td>0</td>
</tr>
</tbody>
</table>

\( a \) The following representative isolates were obtained at 2 h post-CLP: *Bacteroides* spp. (14 isolates), *Lactobacillus fermentum* (2), anaerobic gram-positive rod, and anaerobic gram-negative rod. At 12 h post-CLP, the following representative isolates were obtained: *Bacteroides* spp. (10 isolates), anaerobic gram-positive rods (2 isolates), *Staphylococcus* spp. (2 isolates), and *Escherichia coli*. At the time of death, the following isolates were obtained: *Escherichia coli* (9 isolates), *Enterobacter cloacae* (2 isolates), group D streptococci (3 isolates), aerobic gram-negative rods (2 isolates), *Staphylococcus aureus*, *Corneybac terium* spp., *Bacteroides* spp., and anaerobic gram-negative rod. \( P = 3.9 \times 10^{-4} \) by chi-square analysis.
TABLE 4. Total and coliform CFU per mouse in blood, liver, and spleen of mature and senescent C57BL/6Nia mice at 2 and 12 h post-CLP and at the time of death

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Culture</th>
<th>2 h</th>
<th>12 h</th>
<th>Death</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mature</td>
<td>Senescent</td>
<td>Mature</td>
</tr>
<tr>
<td>Blood</td>
<td>Total</td>
<td>3.99</td>
<td>3.69</td>
<td>3.15</td>
</tr>
<tr>
<td></td>
<td>Coliform</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Liver</td>
<td>Total</td>
<td>7.08</td>
<td>6.40</td>
<td>5.83</td>
</tr>
<tr>
<td></td>
<td>Coliform</td>
<td>3.91</td>
<td>0</td>
<td>3.71</td>
</tr>
<tr>
<td>Spleen</td>
<td>Total</td>
<td>3.99</td>
<td>3.93</td>
<td>3.57</td>
</tr>
<tr>
<td></td>
<td>Coliform</td>
<td>2.47</td>
<td>0</td>
<td>3.37</td>
</tr>
</tbody>
</table>

a Values represent the means obtained from six animals per group.
b Quantitative anaerobic and aerobic plate counts obtained from BHI agar plates.
c Quantitative counts obtained from MacConkey agar plates.
d 0 indicates a quantitative count of <100 bacteria per mouse (below the sensitivity of the method).

DISCUSSION

Age-related defects in acquired immunity have been recognized and extensively documented (19). In vitro observations of the reticuloendothelial system in aging mammals suggest that there are age-dependent alterations of innate function (3, 15). To our knowledge, this is the first investigation that demonstrates an age-dependent increased sensitivity to a bacterial infection that mimics clinically encountered peritonitis. The bacterial kinetics of the CLP model, with respect to quantitative culture of blood, liver, spleen, and cecum, have not been reported previously.

In senescent mice, increased sensitivity to the lethal effects of bacterial peritonitis was manifested by shorter survival times following CLP (MTD of 24.4 versus 38.5 h for mature mice). The factors that determine the length of time animals survive following an acute overwhelming bacterial insult are complex. Endotoxins, cytokines, and other mediators perturb homeostatic responses and contribute to multiple organ system failure and death (2). Lethality studies provide a measurement of the relative sensitivity of a group of animals to a specific stress. In the study reported here animals were subjected to the stress of intraabdominal surgery followed by the stress of bacterial sepsis. It was thought that these stresses might amplify age-related differences in immune function, which could be assessed by differences in bacterial counts of infected tissues.

In bacterial kinetic studies performed on mice that were made septic by CLP, the initial bacterial counts and bacterial species isolated did not differ as a function of the age of the mice. There were no apparent age-related differences in bacterial clearance and killing, as manifested by approximately equal blood, liver, and spleen counts at 2 h. The approximately 10-fold higher liver bacterial count in senescent mice at 12 h compared with that in mature animals suggests that more rapid hepatic clearance in older animals may underlie increased sensitivity to sepsis. Although the difference noted was not significant (P = 0.12), additional studies are underway to examine the possibility. A significant observation in this study was that senescent animals died with a 100-fold lower bacterial burden. It is not known whether the higher bacterial counts found in mature mice resulted from reduced bacterial killing as sepsis progressed or reflected increased bacterial multiplication because of the longer host survival time. We suspect the latter possibility is the case, and it is hoped that work in progress will clarify the point. It is quite possible that no difference in susceptibility to infection per se exists between mice in the two age groups; however, the senescent animals were certainly more sensitive to the effects of sepsis. It is clear from our results that in septic mice with longer survival time, regardless of age, increased numbers of bacteria were found in blood and tissue and were more likely to be coliform organisms (Table 5).

Microgram quantities of endotoxin administered several days prior to CLP have been shown to reduce the postsurgical mortality rate from 81.3% of age-matched controls to 18.8% of pretreated animals (17). Endotoxin-pretreated animals were, thus, more resistant to the lethal effects of bacterial peritonitis. In bacteriologic studies of septic mice following CLP, endotoxin pretreatment reduced the level of bacteria in the bloodstream and peritoneal fluid 100-fold (9). The decrease in total bacterial burden associated with pretreated animals was particularly evident in a reduction in the number of aerobic coliform bacteria (9, 17). In the current investigation, the shorter MTD observed in senescent mice or reflected increased bacterial multiplication because of the longer host survival time. We suspect the latter possibility is the case, and it is hoped that work in progress will clarify the point. It is quite possible that no difference in susceptibility to infection per se exists between mice in the two age groups; however, the senescent animals were certainly more sensitive to the effects of sepsis. It is clear from our results that in septic mice with longer survival time, regardless of age, increased numbers of bacteria were found in blood and tissue and were more likely to be coliform organisms (Table 5).

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TABLE 5. Bimodal distribution within mature and senescent C57Bl/6Nia mice following cecal ligation and puncture: characteristics of the two observed groups

<table>
<thead>
<tr>
<th>Death observed</th>
<th>Blood culture result</th>
<th>Relative quantitative bacterial count</th>
<th>MTD (h) of the following mice:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early</td>
<td>Mixed</td>
<td>Low</td>
<td>Mature</td>
</tr>
<tr>
<td>Late</td>
<td>Pure</td>
<td>High</td>
<td>Senescent</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&lt;27</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&gt;27</td>
</tr>
</tbody>
</table>

FIG. 3. Bimodal distribution at death of total (A) and coliform (B) bacterial counts in blood of mature (■) and senescent (■) mice following cecal ligation and puncture. The bacterial count (log$_{10}$) per mouse is displayed above each bar. Mature mouse 6 survived; therefore, blood counts were not obtained.
and the 100-fold reduction in aerobic coliform bacteria at the
time of death provide strong support for the hypothesis that
senescent mice are more sensitive to the lethal effects of
CLP.
The transition from predominately mixed bacterial sepsis
to pure aerobic coliform sepsis, with increasing host survival
time, was not surprising since that trend has been reported
previously (9, 17). We suspect that the microenvironment
within the ligated, punctured cecum changes over time with
respect to pH, nutrient supply, redox potential, flushing
action of defecation, and other parameters that are inti-
mately involved in the homeostasis of gut bacterial flora (5).
Changes in these parameters may provide strong selective
pressures that favor the multiplication of particular bacteria.
Although a detailed bacteriologic study of the cecal flora
was not performed in this study, we did not observe any age-
related differences in the three predominating colony types
isolated from the cecum at various stages of infection. In the
CLP model, the cecal microenvironmental changes were
manifested by a shift in predominance from anaerobes and
gram-positive bacteria to a predominance of aerobic col-
form bacteria. The strong correlation between cecal isolates
and blood and tissue isolates suggests that the shift in
bacterial species recovered from the blood and reticuloen-
dotheial system tissues as sepsis progressed did not result
from ineffective clearance or killing of resistant organisms.
It is more likely that the bacteria isolated from tissues such as
liver and spleen reflected the same type of bacteria that were
being seeded continuously into the circulation from the
ligated cecum.
In summary, senescent mice were more sensitive to the
lethal effects of CLP, as evidenced by a shorter MTD and
lower bacterial counts at the time of death. By using bacte-
rial counts in blood, liver, and spleen as indirect measures of
innate immune system effectiveness, no age-related defects
were identified between the two groups during the hyper-
or hypodynamic (2 and 12 h, respectively) phases of sepsis.
Blood and tissue isolates reflected a transition within the
ligated cecum from predominately anaerobic bacteria and
gram-positive aerobes to aerobic coliform bacteria. The in-
creased sensitivity of aged mice to invasive bacterial infec-
tion documented in this series of experiments accords
well with human epidemiologic experience and demonstrates
the appropriateness of the model for continued investiga-
tions of sepsis in the aged.

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nold, Jason Hyde, and David McCallum is greatly appreciated.

LITERATURE CITED
defined strains of laboratory animals, part 1, mouse and rat.

Biological handbooks III, p. 45. Federation of American Soci-
eties of Biology. Bethesda, Md.
inflammation and microthrombosis induced by endotoxin, inter-
leukin-1, and tumor necrosis factor and their implication in gram
assessment of the respiratory burst and bactericidal activity of
alveolar macrophages from adult and senescent mice. J. Leu-
kocyte Biol. 43:445–454.
Chem. 4:101–112.
5. Freter, R. 1983. Mechanisms that control the microflora in
the large intestine, p. 32–52. In D. J. Heniges, (ed.), Human
New York.
Anaerobe laboratory manual, 4th ed, p. 145–147. Virginia Poly-
technic Institute and State University, Blacksburg.
statistical method for analyzing mortality data in shock re-
search. Circ. Shock 16:375–381.
1980. Gram negative bacteremia. III. Reassessment of etiology,
epidemiology, and ecology in 612 patients. Am. J. Med. 68:
332–343.
9. McCallum, R. E., K. P. Becker, R. Urbaschek, and B. Urb-
aschek. 1986. Effect of endotoxin pretreatment on the microbial
kinetics of acute peritonitis and sepsis. Circ. Shock 18:337.
metabolism in C3H/HeJ (non-responder) mice during endotoxic
experimental bacteriology. Cambridge University Press, Lon-
don.
and hematological reference values in normal experimental
13. Nichols, R., J. Smith, D. Klein, D. Trunkey, R. Cooper, M.
Adinolfi, and J. Mills. 1984. Risk of infection after penetrating
15. Petrequin, P. R., and A. G. Johnson. 1984. Macrophage activa-
tion by adjuvants in aging mice. J. Leukocyte Biol. 35:251–263.
endotoxin on hepatic glucocorticoid action and glucose metab-
Protective effects and role of endotoxin in experimental septi-
and interleukin-1 as mediators of endotoxin-induced beneficial
and septic shock, a review of laboratory models and a proposal.
of peripheral glucose uptake during sepsis. Arch. Surg. 114:
740–745.
Hall, Englewood Cliffs, N.J.