Synergistic effect of nonspecific immunostimulation and antibiotics in experimental peritonitis

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To assess the role of combined immunomodulator and antibiotic therapy in sepsis, glucan—a beta 1, 3 polyglucose—and gentamicin were administered in a model of murine peritonitis. ICR/HSD mice received one of four treatment regimens: (1) 5% dextrose; (2) gentamicin 0.02 mg intramuscularly (sub-MIC) 2 hours before peritonitis; (3) glucan 0.1 mg intraperitoneally 24 hours before peritonitis; (4) combined glucan-gentamicin treatment. All animals were challenged with 1 x 10⁷ Escherichia coli intraperitoneally. Long-term survival was significantly enhanced in the combined therapy group (56%, p < 0.05) when compared with D,W (0%), gentamicin alone (0%), or glucan alone (9%). Macrophage secretory activity, as assayed by interleukin-1 (IL-1) production, was significantly enhanced by combined therapy when compared with the other three treatment groups. Combined therapy significantly reduced E. coli bacteremia at 8 hours after inoculation, when compared with the other three groups. Availability of host neutrophils was assessed by peripheral counts and bone marrow proliferation assay. Combined glucan-gentamicin significantly enhanced bone marrow proliferation when compared with the other three groups and this enhancement correlated with increased circulating neutrophils. Combined immunomodulator and antibiotic therapy had synergistic effects on survival in E. coli peritonitis. This combined therapy enhanced macrophage secretory activity and bone marrow proliferation. Clinical use of immunomodulators may alter conventional use and dosage of antibiotics.

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In the past decade, investigators have sought to clarify the role of immunomodulators in various models of surgical sepsis. Development of more efficacious and less toxic immunomodulators has increased the likelihood of clinical use of this group of compounds. It is apparent that the clinical use of immunomodulators in the surgical patient with sepsis will be accompanied by appropriate antibiotic therapy. Thus the interaction of these two therapeutic modalities is of critical importance to ensure appropriate therapy. Preliminary investigations have noted possible beneficial effects of combined antibiotic and immunomodulator therapy in several animal models of surgical sepsis.⁵,⁶

Previous studies from our laboratory demonstrated the efficacy of glucan, a β 1,3 polyglucose, in a variety of surgical sepsis models.⁷ Williams et al.⁸ have demonstrated that glucan treatment enhanced long-term survival and maintained reticuloendothelial function in a murine model of Escherichia coli peritonitis. In this latter model, macrophage function appeared to play a key role in the protective effect of glucan. Blockade of macrophage function with methyl palmitate completely reversed the protective effects of the glucan prophylaxis.⁹ While the macrophage may be the key peritoneal host defense effector cell, other studies have noted the importance of the neutrophilic leukocyte in the host immune response to E. coli peritonitis.⁹,¹⁰

Recently investigators have also noted the beneficial
GLUCAN AND E. coli PERITONITIS

Fig. 1. Soluble glucan dose response in mice with E. coli peritonitis. ICR HSD mice were injected intraperitoneally with glucan 24 hours before intraperitoneal challenge with $1 \times 10^4$ E. coli ($n = 25$ to 30 group).

MATERIAL AND METHODS

Mice. Male ICR/HSD mice weighing approximately 18 gm were purchased from Harlan Sprague-Dawley (Houston, Texas). The mice were housed in plastic cages and were fed standard laboratory chow and water ad libitum. The protocols used in this study were reviewed and approved by the Tulane University School of Medicine Advisory Committee for Animal Resources.

Glucan and gentamicin. Soluble glucan was prepared from Saccharomyces cervisiae in accordance with a modification of previously outlined procedures. Dilutions were prepared in sterile pyrogen-free dextrose (5% W/V) and water. Assays for endotoxin with use of the limulus lysate procedure were routinely negative for the glucan preparations. Gentamicin sulfate was purchased from Sigma Chemical Co. (St. Louis, Mo.).

Bacteriologic studies. For intraperitoneal challenge, E. coli was prepared as previously described. E. coli bacteremia was determined in control and experimental mice by sampling tail blood at various periods after E. coli challenge. Peritoneal cultures were obtained by lavaging the peritoneal cavity with 10 ml sterile saline solution. Techniques of bacterial culture have been previously described.

Experimental protocol. ICR mice were placed into one of four treatment groups ($n = 25$ to 34 per group). Group 1 ($n = 25$) received 5% dextrose injections. Group 2 ($n = 30$) received gentamicin 0.02 mg intra-
Fig. 2. Gentamicin sulfate dose response in mice with experimental E. coli peritonitis. ICR, HSD mice were injected intramuscularly with gentamicin 2 hours before intraperitoneal challenge with $1 \times 10^8$ E. coli (n = 20 to 25/group).

Gentamicin AND E. coli PERITONITIS

<table>
<thead>
<tr>
<th>Gentamicin (mg/kg)</th>
<th>Percent Survival</th>
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<tr>
<td>100</td>
<td>100</td>
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<td>80</td>
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<td>5</td>
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<td>3</td>
<td>30</td>
</tr>
<tr>
<td>0.8</td>
<td>20</td>
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<tr>
<td>0.6</td>
<td>10</td>
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</table>

ICR mice

$1 \times 10^8$ E. coli 1P

Muscularly 2 hours before E. coli inoculation. Group 3 (n = 31) received soluble glucan 0.1 mg intraperitoneally 24 hours before E. coli inoculation. Group 4 (n = 34) received combined glucan and gentamicin therapy. All mice were challenged with $1 \times 10^8$ E. coli intraperitoneally and followed for long-term survival. Both peritoneal and blood cultures were obtained in all groups at 8 and 12 hours after E. coli inoculation. Other groups of mice were killed before challenge for collection of splenic macrophages and bone marrow cells. Each set of experiments was performed at least twice.

Determination of peripheral leukocyte and neutrophil levels. Total peripheral leukocytes were determined by use of the Unopette white blood cell dilution system (Fisher Scientific, Houston, Texas). Twenty microliters of blood was obtained from the tail of mice at 9 and 12 hours after E. coli inoculation. The blood was subsequently transferred to vials containing white blood cell diluting fluid and the cells were counted on a hemocytometer.

Peripheral blood was also collected on glass slides, stained for 8 minutes with Wright's stain (Sigma Chemical Co.), and buffered with sterile distilled water (pH 7.0) for 5 minutes. The slides were examined under oil immersion (100X) with a light microscope and were subjected to three separate microscopic screenings of 100 cells each.

Interleukin-1 production by splenic macrophages. Splenic macrophages were isolated by aseptically excising and mincing the spleens of mice. The resulting splenocyte preparation was filtered and centrifuged, and erythrocytes were removed by hypotonic lysis. Splenocytes were then resuspended in RPMI-1640 media (5 x 10^6 cells/ml) supplemented with fetal bovine serum and incubated for 4 hours to allow macrophage adherence. The cultures were washed three times to remove nonadherent cells and medium was replenished over the adherent cell population at a final concentration of 2 x 10^6 cells per ml. The resulting cultures were incubated for 24 hours (37°C and 5% CO_2), the supernatant was recovered and centrifuged to remove cells.

Interleukin-1 activity was evaluated by thymocyte proliferation according to the method of Oppenheim et al. Thymocytes (1 x 10^5 per well) isolated from C57BL/6J mice were coincubated with macrophage culture supernatant in 96-well microtitre plates for 72 hours in the presence of 0.1 µg per well of phytohemagglutinin (PHA). The cultures were pulse-labeled with
tritiated thymidine (1 μCi per well) for the final 8 hours of the incubation period. Purified IL-1 (Collaborative Research, Lexington, Mass.) was incubated with thymocytes in the presence of PHA (1 μg per well) at IL-1 concentrations ranging from 0.1 to 20 U/ml to generate a standard curve for IL-1 activity. The linear portion of the curve was used to quantitate IL-1 levels.

In vitro bone marrow proliferation. Bone marrow cells were obtained at the time of E. coli inoculation and 9 hours postinoculation by excising the femur of the ICR mice and flushing the marrow cavity with 6 ml of medium. The bone marrow cell suspension was collected, washed, centrifuged, and diluted to 1 x 10^7 cells per milliliter. Bone marrow cells were then incubated in 96-well microtiter plates (1 x 10^5 cells per well) for 40 hours at 37° C in 5% CO₂. One microcurie of tritiated thymidine was added for the final 8 hours of incubation. Tritiated thymidine uptake was determined as an index of bone marrow proliferation.

Statistics. Statistical comparison between groups was performed with use of the Student T test. A value of p < 0.05 was considered significant. Statistical analysis of survival was based on χ² with 1 degree of freedom. A 95% confidence level was considered significant. In addition, one-way analysis of variance was used when appropriate.

RESULTS

Survival. Preliminary studies in murine E. coli peritonitis established the appropriate doses for glucan (4 mg/kg) and gentamicin (0.8 mg/kg). Glucan or gentamicin at the doses indicated resulted in 9% survival (Figs. 1 and 2). This dose of gentamicin exhibited no in vitro inhibition of E. coli cultures.

The majority of deaths in the experimental groups occurred 12 to 24 hours after E. coli challenge. There were no deaths after 48 hours. The combined glucan-gentamicin therapy resulted in a 56% long-term survival rate—a significant improvement (p < 0.05) when compared with the other three treatment regimens (Fig. 3).

Peritoneal bacterial counts. At 8 hours postinoculation, all three treatment regimens showed decreased E. coli levels when compared with the dextrose control.
Table I. Decreased E. coli levels in the peritoneal cavity after treatment with glucan-gentamicin

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Colony-forming units (x 10^9)</th>
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<tbody>
<tr>
<td></td>
<td>8 hr</td>
</tr>
<tr>
<td>Dextrose control</td>
<td>96.7 ± 4.8</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>2.3 ± 1.1*</td>
</tr>
<tr>
<td>Glucan</td>
<td>0.6 ± 0.4*</td>
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<tr>
<td>Glucan and</td>
<td>0.2 ± 0.1*</td>
</tr>
<tr>
<td>gentamicin</td>
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ICR: HSD mice were injected with D.W., gentamicin 0.02 mg IM, glucan 0.1 mg IP, or glucan and gentamicin. Glucan was administered 24 hours before and gentamicin 2 hours before IP challenge with 1 × 10^8 E. coli. Peritoneal cultures were performed at 8 hours and 12 hours after inoculation. *p < 0.05 compared with dextrose controls. **p < 0.05 compared with gentamicin alone.

Table II. Suppression of E. coli bacteremia after peritonitis

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Colony-forming units/ml blood (x 10^8)</th>
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<tbody>
<tr>
<td></td>
<td>8 hr</td>
</tr>
<tr>
<td>Dextrose control</td>
<td>4.25 ± 2.4</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>7.43 ± 4.7</td>
</tr>
<tr>
<td>Glucan</td>
<td>0.68 ± 0.3*†</td>
</tr>
<tr>
<td>Glucan and</td>
<td>0.05 ± 0.02*‡</td>
</tr>
<tr>
<td>gentamicin</td>
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</tbody>
</table>

ICR: HSD mice were injected with D.W., gentamicin 0.02 mg IM, glucan 0.1 mg IP, or glucan and gentamicin. Glucan was given 24 hours and gentamicin 2 hours prior to 1 × 10^8 E. coli IP. Tail blood samples were obtained at 8 and 12 hours and plated on devoxycholate agar. *p < 0.05 compared with dextrose control. **p < 0.05 compared with gentamicin alone. †p < 0.05 compared with glucan alone.

group (Table I). In addition, the combined glucan and gentamicin therapy significantly (p < 0.05) reduced E. coli numbers when compared with the gentamicin-only treatment. At 12 hours the results were similar with the three treatment regimens significantly (p < 0.01) decreasing E. coli numbers when compared with the dextrose control. While combined treatment significantly (p < 0.05) decreased E. coli levels when compared with the gentamicin treatment alone, there was no significant difference between the glucan treatment and the combined therapy groups.

E. coli bacteremia. Peripheral blood cultures revealed a significant decrease in E. coli bacteremia at 8 hours after challenge with the combined glucan plus gentamicin treatment (Table II). Glucan treatment alone significantly diminished the E. coli numbers at 8 hours when compared with the dextrose control or gentamicin-only groups. Moreover, the combined therapy resulted in a further significant reduction when compared with the glucan alone. At 12 hours after E. coli inoculation, gentamicin alone and glucan plus gentamicin resulted in significant reductions in E. coli levels.

Macrophage IL-1 production. Macrophages from mice treated with 5% dextrose or gentamicin alone produced no IL-1 (Fig. 4). Macrophages from animals treated with glucan alone demonstrated a significant increase in IL-1 production when compared with the former two groups. However, macrophages from the combined (glucan plus gentamicin) group exhibited a significantly greater IL-1 production than the animals treated with glucan alone (p < 0.001).

In vitro bone marrow proliferation and peripheral leukocyte counts. Combined glucan-gentamicin therapy significantly enhanced bone marrow proliferation over control treatment at the time of inoculation and at 9 hours after inoculation (85% and 220%, respectively) (Table III). In addition, at the time of inoculation the glucan-gentamicin group exhibited significantly (p < 0.05) greater in vitro bone marrow proliferation than either regimen alone, as shown by a 21% increase when compared with glucan and a 29% increase when compared with gentamicin. Similarly, at 9 hours post-inoculation the combined therapy group demonstrated greater proliferation than glucan (86% increase) or gentamicin alone (26% increase) (p < 0.01).

Peripheral leukocyte counts correlated with bone marrow assay, with combined treatment resulting in significantly higher counts than the dextrose controls or gentamicin treatment alone (Table IV). Both combined glucan-gentamicin and glucan-alone groups had markedly elevated neutrophil counts when compared with dextrose controls or animals given gentamicin alone.

DISCUSSION

This study reports a synergistic effect when combined immunomodulator (glucan) and antibiotic (gentamicin) are used in a murine model of E. coli peritonitis. Lahnborg et al. have reported similar results with use of glucan and benzylpenicillin in an ileal exclusion and devascularization model of peritonitis in the rat. Polk et al. used a suture that was saturated with Klebsiella pneumoniae and placed in the adductor muscle mass of the mouse in evaluating muramyl dipeptide (MDP) and antibiotics. In this model, if MDP was added before infection, an additive...
effect on survival was obtained with both chloramphenicol and cephaloridine. However, when MDP treatment was delayed until after onset of infection, no long-term beneficial effect was noted. Similarly, Frazier-Smith and Matthews reported the use of an MDP analog in a Pseudomonas peritonitis model. They noted that MDP given daily for 4 days before the infection and gentamicin given 2 and 4 hours postinfection resulted in a synergistic effect on survival. However, modifying the dose of the MDP analog so that the animal received less than 4 daily doses resulted in significant decreases in survival. Moreover, the MDP analog given after infection had no effect on survival.

Our studies clearly demonstrate a synergistic effect on survival in murine peritonitis when a single dose of glucan was given 24 hours before infection and a single dose of gentamicin was given 2 hours before E. coli inoculation. While glucan treatment after E. coli inoculation was not given in these experiments, preliminary data from our laboratory suggest that glucan treatment after E. coli inoculation has no beneficial effect on long-term survival.

Recent studies by Dunn et al. in a rat model of E. coli peritonitis have demonstrated that both macrophages and polymorphonuclear leukocytes appeared to be major contributors to host defense in peritonitis. Their studies reveal that immediately after peritoneal inoculation with E. coli, macrophages were numerically predominant. However, 2 hours after infection, a

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**Figure 4.** Macrophage IL-1 production from the various treatment groups. Combined glucan-gentamicin therapy resulted in significant enhancement in macrophage IL-1 secretion (n = 16/group).

**Table III.** Bone marrow proliferation in E. coli peritonitis: Effect of glucan-gentamicin

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Tritiated thymidine uptake (CPM)</th>
<th>9 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextrose control</td>
<td>3160 ± 75</td>
<td>543 ± 33</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>4493 ± 369*</td>
<td>1383 ± 101*</td>
</tr>
<tr>
<td>Glucan</td>
<td>4700 ± 263*</td>
<td>916 ± 191*</td>
</tr>
<tr>
<td>Glucan and gentamicin</td>
<td>5787 ± 385*§§</td>
<td>1173 ± 123*§§</td>
</tr>
</tbody>
</table>

n = 8-12/group.

*p < 0.05 when compared with dextrose control.

**Table IV.** Effect of glucan-gentamicin on peripheral leukocytes and neutrophils in E. coli peritonitis (9 hours after challenge)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Leukocytes (×10^6)</th>
<th>Neutrophils (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextrose control</td>
<td>10.3 ± 2.8</td>
<td>14 ± 2</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>11.1 ± 1.7</td>
<td>30.6 ± 12</td>
</tr>
<tr>
<td>Glucan</td>
<td>15.1 ± 2.1</td>
<td>63 ± 4.5§</td>
</tr>
<tr>
<td>Glucan and gentamicin</td>
<td>19.7 ± 2.9§§</td>
<td>63.7 ± 10§§</td>
</tr>
</tbody>
</table>

n = 6/group.

*p < 0.01 when compared with dextrose control.

§§p < 0.01 when compared with gentamicin alone.
neutrophil influx began; this was related to the size of the peritoneal inoculum. Further studies demonstrated that phagocytic activity of individual neutrophils and macrophages was similar. Thus because of their greater number, peritoneal macrophages appear to be the major initial host response in peritonitis. However, neutrophils, with increasing numbers over the ensuing 24 to 48 hours, also contribute to the host defenses in peritonitis.\(^8\)\(^{13}\)

Recent studies of murine \(E. \text{coli}\) peritonitis in our laboratory have demonstrated that, in addition to enhanced macrophage function, glucan treatment resulted in increased peritoneal and peripheral neutrophils.\(^7\) In addition to increased numbers of neutrophils, glucan was shown to enhance phagocytic function of these cells.\(^7\) It is apparent that both the peritoneal macrophage and the neutrophil are central elements of the host defense against peritoneal infection.

Our study demonstrated enhanced macrophage secretory activity following glucan administration, as manifested by enhanced IL-1 production from splenic macrophages. There are several beneficial effects of IL-1 that may contribute to increased survival in this model. Interleukin-1 is a potent stimulus of acute-phase protein synthesis. These proteins include haptoglobin, ceruloplasmin and C-reactive protein.\(^16\) These compounds may have a beneficial effect on host immunity. For example, C-reactive protein has been demonstrated to enhance complement function and improve survival in streptococcal pneumonia in mice.\(^16\) Interleukin-1, through release of interleukin-2, can also affect host lymphocyte function.

In addition, IL-1 may also contribute to protection in \(E. \text{coli}\) peritonitis through its effects on neutrophil function. This monokine has been reported to increase the percentage of nitroblue tetrazolium-positive neutrophils and enhance neutrophil chemotaxis, which suggests an increased capacity for killing of bacteria.\(^7\)\(^{18}\) Moreover, IL-1 has been demonstrated to selectively increase the release of neutrophils from the bone marrow.\(^19\) Another intriguing property of IL-1 is its effect on transport and storage of metals (such as iron) in the liver. The net effect is to decrease plasma iron levels, which has been correlated with decreased pathogenicity of bacteria.\(^18\) It is of interest that the addition of gentamicin resulted in increased IL-1 production in the glucan-stimulated macrophages, which indicates possible interactions at the macrophage level.

The apparent beneficial effect of gentamicin at sub-MIC doses may be due to its effects on the host immune response to \(E. \text{coli}\) peritonitis. Previous studies have documented the effects of gentamicin on both macrophage and neutrophil function.\(^10\)\(^{12}\) Andreana et al.\(^{10}\) used an isolated rat liver model to evaluate reticuloendothelial function and phagocytic removal of \(E. \text{coli}\) from the blood. They noted that incubation of \(E. \text{coli}\) with gentamicin (1/5 of the MIC) resulted in increased phagocytosis and killing of the bacterium.\(^10\) Haranaka et al.\(^{12}\) demonstrated a synergism between gentamicin and antibody to \textit{Pseudomonas} species in a murine model of peritonitis. A combination of gentamicin plus \textit{Pseudomonas} antibody markedly enhanced bactericidal activity of peritoneal macrophages. This enhancement occurred even in animals stimulated with \(C. \text{parvum}\), which indicates a supplemental effect of gentamicin on enhanced macrophage function.\(^12\)

McDonald et al.\(^{11}\) have shown that gentamicin affects neutrophil function. When exposed to gentamicin \(E. \text{coli}\) was more susceptible to killing by enzyme systems of the neutrophil than control bacteria. However, phagocytic uptake of the \(E. \text{coli}\) did not appear to be affected by previous incubation with the gentamicin.\(^11\) In our report, it appears that the combined glucan-gentamicin treatment had synergistic effects on survival by decreasing bacterial counts in both the peritoneal cavity and the peripheral blood. The combined treatment significantly decreased \(E. \text{coli}\) bacteremia at 8 hours when compared with either glucan or gentamicin alone. While further studies are needed to precisely elucidate mechanisms of protection at the macrophage and neutrophil level, it is interesting to speculate that effects of the sub-MIC dose of gentamicin may have allowed more efficient phagocytosis and killing by the host immune defenses.

Combined glucan-gentamicin therapy resulted in a marked enhancement in bone marrow proliferation, with subsequent improved survival. This is not surprising since cellular elements such as the neutrophil have been described as an important cellular defense mechanism in bacterial peritonitis.\(^3^\)\(^{14}\) Low-dose glucan treatment in mice has been shown to markedly enhance the numbers of both pleuripotent hematopoietic stem cells and granulocyte-macrophage progenitor cells,\(^20\) which may contribute to increased cellular elements in response to \(E. \text{coli}\) peritonitis. Similar data with gentamicin have been reported.\(^21\) While high doses of gentamicin are suppressive to bone marrow elements, extremely low doses (0.5 \(\mu\)g/ml) have been shown to enhance proliferation of bone marrow macrophage progenitor cells.\(^21\) These findings confirm the report of Honma et al.\(^{13}\) However, the mechanism at present is unknown, and further studies are needed to elucidate this phenomenon. In this study, the combination of
glucan and gentamicin significantly enhanced marrow proliferation over either treatment alone. In this study, peripheral leukocyte and neutrophil counts correlated well with bone marrow proliferation. Availability of circulating neutrophils due to enhanced bone marrow function may increase host defense against sepsis, which thus explains one protective effect of current antibiotic therapy.

Availability of circulating neutrophils due to enhanced mechanism of the combined glucan-gentamicin proliferation over either treatment alone.

Available number of circulating neutrophils due to enhanced mechanism of the combined glucan-gentamicin proliferation over either treatment alone.

Results from this series of experiments demonstrates that sub-MIC doses of gentamicin can affect aspects of host immunity, especially when used in conjunction with an immunomodulator. Thus clinical use of immunomodulators may require that antibiotic dosages be modified to obtain maximum bactericidal effect as well as maximal host immune response. These considerations may have a major impact on toxicity and cost-effectiveness of current antibiotic therapy.

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DISCUSSION

Dr. Ronald Busuttii (Los Angeles, Calif.). You suggested the possible correlation of your data to future clinical studies. However, in your studies you administer the agents before bacterial challenge. Administration after bacterial challenge would be much more likely to stimulate the clinical situation. Do you have any data to support a beneficial effect if immunomodulators are given after the infection?

Dr. Christopher Baker (New Haven, Conn.). What do you think the mechanism of interleukin-1 release is? Have you thought about using C3A, since it has been suggested that the effect in HIG mice might be failure to produce prostaglandin E2 in response to endotoxin. I believe that we have to be careful about viewing all increases in interleukin-1 activity as good. I think there are levels beyond which serious deleterious effects occur. We have data on burn animals to show that.

Dr. Thomas Stellato (Cleveland, Ohio). Can you comment on the specificity of the glucan and the antibiotic for the Escherichia coli? We looked at this combination in a fibrin clot model and were able to demonstrate that we could sterilize fibrin clots laden with bacteriodes but not with E. coli. Do you have any information about that?

Dr. Mark Malanganii (Louisville, Ky.). Could you speculate on what findings you would have if you increased the
dose of either the gentamicin or the glucan to try various combinations to increase survivorship above 56%? One of the more interesting aspects of your study is the bone marrow proliferation, which if you believe that macrophages originate from stem cells in the bone marrow might actually show that over time you may have another way to modulate interleukin-1 production.

Dr. Richard Gamelli (Burlington, Vt.). Have you looked at what is going on in the spleen? In a mouse, the spleen is going to be a much more critical site for hematopoietic activity. The spleen may well undergo not a percentage increase but a logarithmic increase not only in its activity but also in its size.

Dr. Browder (closing). Dr. Busuttil, you touched on the major argument whenever any of these compounds are studied. The model presented today is one of an acute septic episode. I personally know of no immunomodulator that has been shown to be very effective when administered after the onset of an acute septic episode such as this one. In this particular model, glucan given 2 hours after the E. coli inoculation prolonged survival about 48 hours, but we observed no beneficial effect on long-term survival, as we saw with the pre-peritonitis treatment.

Dr. Baker, we have not used this model in other animals. I believe that obviously the present study is just a starting point with which to look at protective mechanisms in more depth. I would agree with you that interleukin-1 is not necessary "all good." We use this assay primarily as a marker to determine macrophage stimulation. We have found that it is a very accurate predictor in that regard after glucan therapy.

Dr. Siellato, we have not used glucan in peritonitis models with other organisms. We have used it in various other sepsis models such as Candida albicans and Staphylococcus, but these were intravenous injections, and we have not really looked at other peritonitis models in that regard.

Dr. Malangoni, if you increase the dose of either glucan or gentamicin you will improve survival. Let me emphasize that this model is a very artificial, structured protocol. We constructed it to study possible interactions with the host immunity of the immunomodulator and the antibiotic. I think that we now need to analyze these principles and use them in possibly more long-term models of sepsis.

Dr. Gamelli, we have not looked at splenic function or the spleen in this particular model. Our earlier work with the particulate glucan demonstrated a profound effect on the spleen, with increased size and number of cells. We have reported that in some detail, but we have not studied the soluble glucan presented today in that regard.