

Protective Effect of Glucan Against Systemic *Staphylococcus aureus* Septicemia in Normal and Leukemic Mice

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The reticuloendothelial stimulant glucan, a beta-1,3-polyglucose component of the cell wall of *Saccharomyces cerevisiae*, was evaluated for its ability to modify *Staphylococcus aureus*-induced lethality in normal and leukemic mice. In normal mice the intravenous injection of glucan (0.45 mg per mouse) 7 and 4 days prior to intravenous challenge with *S. aureus* (1.0×10^9) resulted in a significantly increased survival. Histological examination of the kidneys revealed that glucan significantly inhibited renal necrosis associated with systemic staphylococcal diseases. Further studies indicated that glucan administration not only enhanced survival of leukemic mice, but also increased survival of leukemic mice with experimentally induced staphylococcal septicemia. These data denote that glucan enhances nonspecific resistance to *S. aureus* sepsis, promotes survival during leukemic episodes, and increases survival time of leukemic mice with experimentally induced staphylococcal infection.

Previous studies have demonstrated glucan, a component of the cell wall of *Saccharomyces cerevisiae*, to be a potent stimulant of the reticuloendothelial system (6, 16). The intravenous administration of glucan to mice results in hypertrophy of the major reticuloendothelial organs as well as a concomitant increase in activation and proliferation of macrophages (18, 19, 20). The hyperfunctional state of the reticuloendothelial system induced by glucan is associated with a marked enhancement of cellular and humoral immunity (18, 19). Glucan has been demonstrated to promote increased resistance to tumor growth in an allogenic rat tumor (7) and in two syngenic mouse tumors (4). Preliminary clinical studies have also indicated that intralesional injection of glucan into human subcutaneous metastatic lesions results in the accumulation of activated macrophages, prompt tumor cell necrosis, and lesion regression (14, 15).

In view of glucan's diverse immunological and antitumor activity, experiments were undertaken to evaluate whether glucan administration would modify experimentally induced bacterial sepsis in normal and leukemic mice. In recent years, infection due to saprophytic or opportunistically pathogenic microorganisms has become a significant clinical problem (12). These infections are particularly prevalent in patients who are immunodeficient, due to the malignant state or immunosuppressive therapy (11). The gram-positive opportunistic pathogen *Staphylococcus aureus* has been increasingly implicated as a

cause of secondary infection (11) and was, therefore, chosen as the infectious model for this study. The ability of glucan to enhance nonspecific protection against *S. aureus* septicemia in normal and leukemic mice was initially ascertained.

MATERIALS AND METHODS

Animals. AKR/J male mice were obtained from Jackson Laboratory, Bar Harbor, Maine. The animals were housed in plastic cages and were fed Purina Laboratory Chow and water ad libitum.

Glucan. Glucan was prepared by a modification of the method of Hassid et al. (9). Dilutions were made in physiological saline (0.9% wt/vol) to provide 2.25 mg/ml for intravenous injection. Isovolumetric saline served as the control.

Bacteria. A clinical isolate of *S. aureus* was obtained from the Tulane Medical Center Hospital. The organism was subcultured in Trypticase soy broth for 18 h at 37°C in a shaking water bath (50 rpm). Identity was verified by biochemical tests and purity by streak plating on blood agar. Isolated colonies were subcultured to Trypticase soy agar slants and maintained at 4°C. For each experiment, *S. aureus* was subcultured in Trypticase soy broth for 18 h at 37°C in a shaking water bath (50 rpm). The culture was centrifuged ($2,000 \times g$) for 15 min, and the cell pellet was washed three times with phosphate-buffered saline (0.9% wt/vol). Cell numbers were determined at 24 h in triplicate on Trypticase soy agar.

Leukemia. AKR mice with lymphocytic leukemia (BW 5147) were obtained from Jackson Laboratory. The leukemia tumor was maintained by the subcutaneous injection of 10^6 tumor cells into 20-g AKR male mice. The cells for each experiment were prepared by

culturing AKR leukemic cells. Tumor cell suspensions were subcultured into 25-cm² Falcon tissue culture flasks (Becton Dickinson Co., Oxnard, Calif.) containing RPMI 1640 medium (Microbiological Associates, Walkersville, Md.) with 10% (vol/vol) fetal calf serum (KC Biological Inc., Lexena, Kan.), 2% (vol/vol) penicillin-streptomycin (Microbiological Associates), 0.1 mg of gentamicin (Schering Corp., Kenilworth, N.J.) per ml, and 1% (vol/vol) amphotericin B (Grand Island Biological Co., Grand Island, N.Y.). After incubation for 4 days at 37°C and 5.0% CO₂ tension, the culture was centrifuged (1,000 × g), and the cell pellet was washed three times in RPMI 1640 without antibiotics or antifungal agents. Cells were counted on a hemacytometer, and viability was determined by trypan blue exclusion. Dilutions were made in RPMI 1640 with no antibiotics or amphotericin B.

Experimental procedures. In the initial bacterial response study, groups of mice were injected either with glucan (1.0 mg per mouse) or isovolumetric saline on days 7 and 4 prior to challenge with $1.0 \pm 0.25 \times 10^9$ viable *S. aureus*.

In secondary infection studies, AKR lymphocytic leukemic cells (10^6) were injected intravenously into all mice on day 0. Groups of mice were given glucan (0.45 mg per mouse) or saline intravenously on day 0, 1, 2, and 5 and thereafter on alternate days up to day 27. Peripheral leukocyte counts were employed to monitor the onset of lymphocytic leukemia. On day 13, one-half of the populations of each group were challenged intravenously with $1.0 \pm 0.25 \times 10^9$ *S. aureus*. All groups were monitored daily for survival.

Histology. Samples of liver, lung, spleen, kidney, and brain were taken on day 12 from glucan-pretreated and saline control animals which were injected with *S. aureus*. All samples were fixed in 10% (vol/vol) Formalin. The sections were stained with hematoxylin-eosin.

Statistics. Statistical comparisons between groups were performed employing Student's *t* test. A value of $P < 0.05$ was considered significant. Statistical analyses of survival curves were based on chi-square with

one degree of freedom. A chi-square value representing 95% confidence level was considered significant. All chi-square values represent a comparison between a treatment group and its appropriate control.

RESULTS

Effect of glucan on susceptibility of mice to *S. aureus*. To evaluate the protective effect of glucan against systemic staphylococcal infection, glucan was administered intravenously 7 and 4 days prior to intravenous challenge with *S. aureus*. At a challenge dose of 10^9 *S. aureus*, a 30% mortality was noted by day 1 in the saline control group (Fig. 1). In contrast, only 3% mortality was observed in the glucan-pretreatment group in an equivalent time period. The median survival time for the saline control group was approximately 1.6 days as compared to 14 days for the glucan-pretreatment group. A 100% mortality was observed at 14 days in the saline control group, at which time 60% of the glucan-pretreated mice were still alive. The glucan-pretreatment group did not show 100% mortality until day 26.

Influence of glucan on the development of leukemia. Systemic lymphocytic leukemia was induced by intravenous administration of 10^6 cultured AKR leukemic cells. Peripheral leukocyte counts were employed to monitor the onset of the malignant episode (Fig. 2). Within 8 days, the saline control group showed a leukocyte count of approximately 12,000/mm³ ($P < 0.01$) (Fig. 2). In contrast, the glucan-treated group did not show a comparable leukocyte count until day 13. By day 16, the peripheral leukocyte count in the glucan group was approximately 14,000/mm³. In contrast, the saline control group had a peripheral leukocyte count of

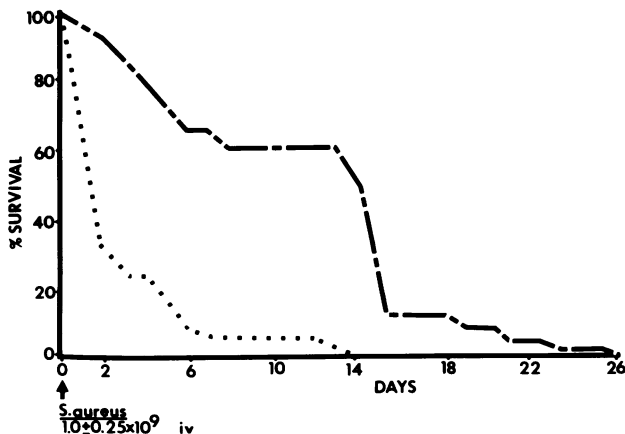


FIG. 1. Glucan (1.0 mg per mouse) enhanced survival of mice to intravenous challenge with $1.0 \pm 0.25 \times 10^9$ viable *S. aureus*. Glucan (— —) was administered intravenously 7 and 4 days prior to challenge with 10^9 *S. aureus*. Isovolumetric saline (· · ·) served as the control in *S. aureus*-injected mice. $N = 46$ per group.

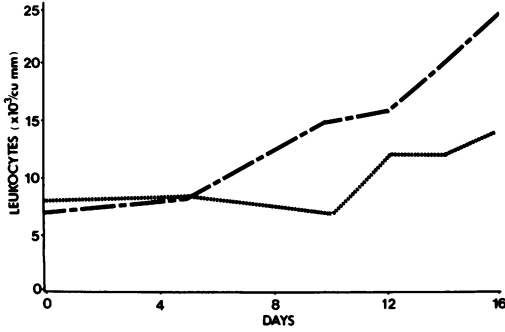


FIG. 2. Comparative alterations in blood leukocytes of AKR mice injected with 10^6 lymphocytic leukemic cells and subsequently treated with intravenously administered glucan (—) or saline (---). $N = 15$ per group.

25,000/mm³ ($P < 0.01$) in an equivalent time period. Although both the saline control and glucan group showed an increase in peripheral leukocyte count, there was a marked disparity in the rate of increase.

Effect of glucan on systemic staphylococcal disease in leukemic mice. Glucan, administered in the dose of 0.45 mg per mouse on days 0, 1, 2, and 5 and thereafter on alternate days up to day 27, resulted in a modification of survival patterns of leukemic mice (Fig. 3) as well as leukemic mice challenged intravenously on day 13 with 10^9 *S. aureus* (Fig. 4). By day 20, leukemic mice that received glucan manifested an 80% survival. However, the saline control group showed only 50% survival in an equivalent time period. At day 25, the glucan group showed 70% survival as compared to 20% in the saline control group (Fig. 3).

Leukemic mice that received glucan prior to and following the administration of *S. aureus* manifested a 90% survival on day 20. In contrast, the saline control mice showed only 10% survival in an equivalent time period. The median survival time in the control group was 15 days, in contrast to 21 days in the glucan-treated group. By day 25, the glucan group showed 30% survival, while all of the saline control mice with lymphocytic leukemia and experimentally induced staphylococcal secondary infection had succumbed by day 23 (Fig. 4). The effectiveness of glucan in enhancing survival of mice injected with lymphocytic leukemia (Fig. 3) was significantly reduced when the mice received a subsequent injection of *S. aureus* (Fig. 4).

Histological observations. Histological examination of glucan-pretreated and saline control animals on day 12 following intravenous injection of 10^9 *S. aureus* revealed marked pathological changes in the kidneys. There was a

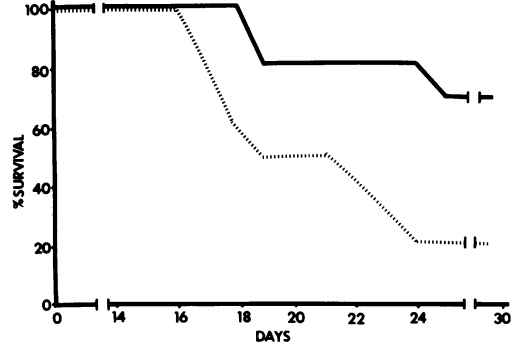


FIG. 3. Effect of glucan administration on survival of AKR mice following administration of 10^6 syngenic lymphocytic leukemic cells. Glucan (—) or saline (· · · · ·) was administered intravenously on days 0, 1, 2, and 5 and thereafter on alternate days to day 27. $N = 15$ per group.

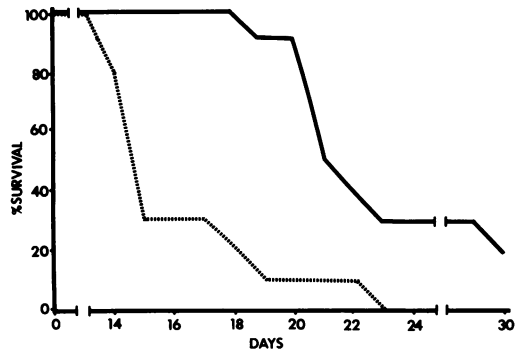


FIG. 4. Glucan-induced enhancement in survival of AKR mice which received 10^6 lymphocytic leukemic cells on day 0 and a subsequent intravenous injection of *S. aureus* (10^9) on day 13. Glucan (—) or saline (· · · · ·) was intravenously administered on days 0, 1, 2, and 5 and on alternate days up to day 27. The increased susceptibility of leukemic mice to intravenous *S. aureus* can be ascertained by comparison of the mortality patterns of normal mice (Fig. 3). The experiments depicted in Fig. 3 and 4 were performed simultaneously. $N = 15$ per group.

diffuse acute pyelonephritis in the renal cortex of saline control mice (Fig. 5). Extensive necrosis of renal parenchyma was observed as a consequence of abscess formation (Fig. 6). There was also a marked dilation of renal tubules, which were filled with inflammatory cellular elements and necrotic debris. In contrast to the severe degenerative changes in control mice, the kidneys of glucan-treated mice exhibited relatively minimal pathological changes due to the acute septic episode (Fig. 7). The livers of glucan-treated mice manifested the typical dose-dependent granulomatous reaction characterized by a predominant monocytic infiltrate (Fig. 8).

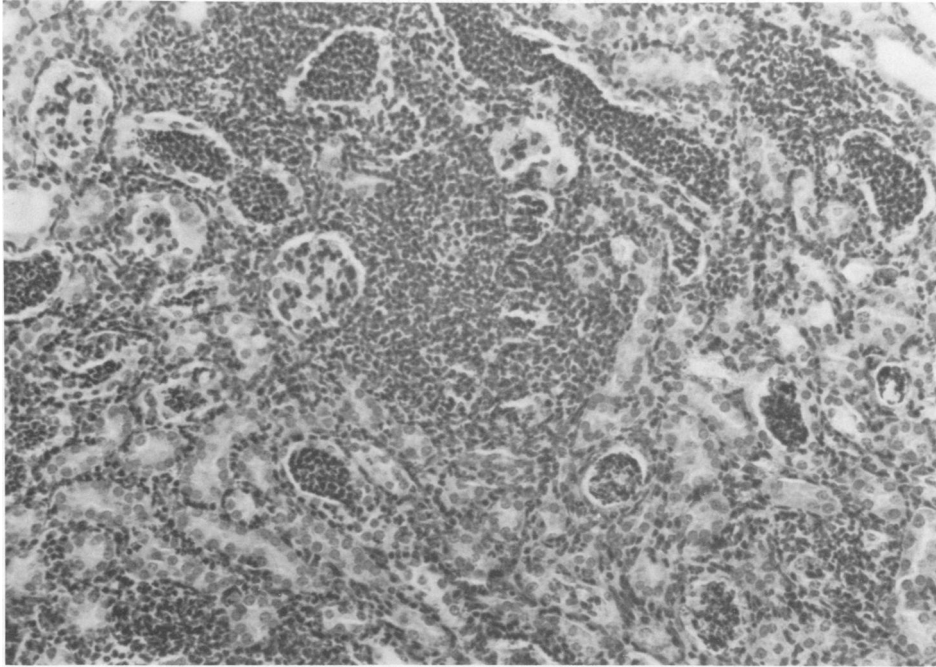


FIG. 5. *Kidney of saline control mouse on day 12, showing a diffuse acute pyelonephritis in the renal cortex, characterized by necrosis of renal parenchyma and a chronic inflammatory cell infiltrate. Early abscess formation was observed along with a marked dilation of renal tubules filled with inflammatory elements and necrotic debris ($\times 200$).*

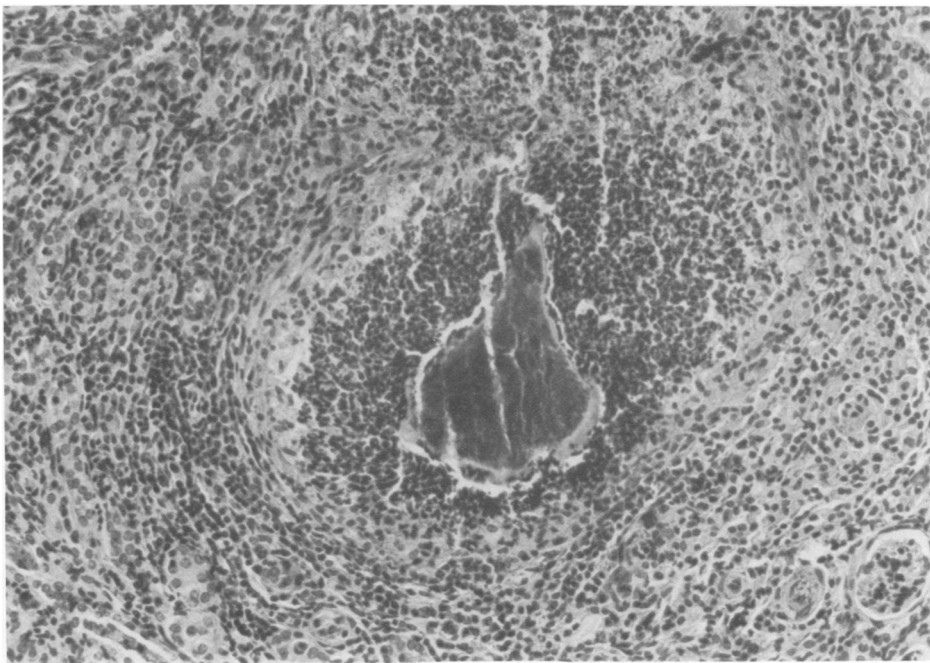


FIG. 6. *Abscess formation in the kidney of a saline control animal on day 12. Extensive necrosis of renal parenchyma was observed as a consequence of abscess formation ($\times 200$).*

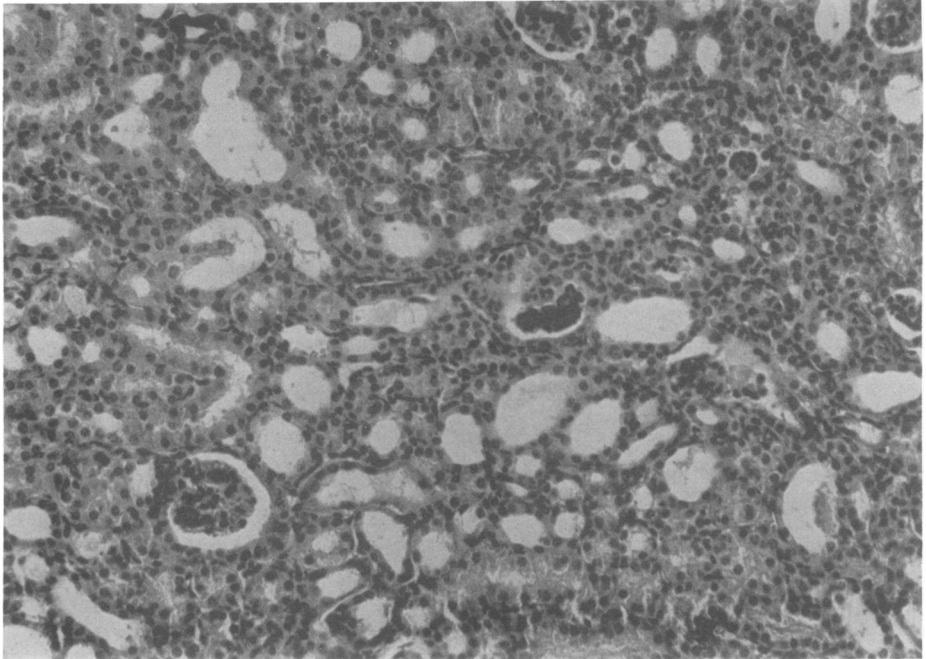


FIG. 7. *Kidney of a glucan-pretreated mouse on day 12 after S. aureus administration, showing minimal pathological changes along with a maintenance of renal integrity. ($\times 200$)*

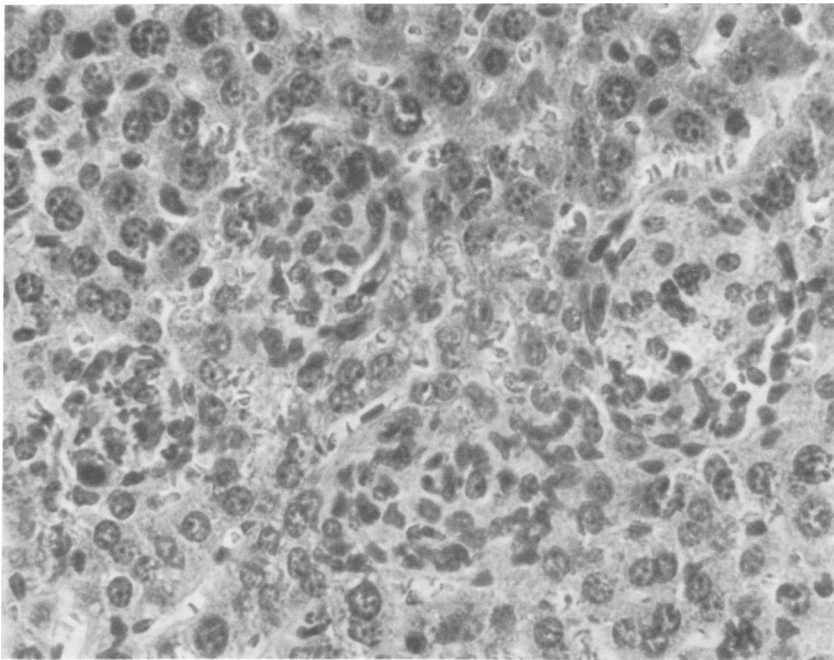


FIG. 8. *Liver of a glucan-pretreated mouse 12 days after S. aureus challenge, manifesting the typical granulomatous reaction characterized by a predominant monocytic infiltrate. ($\times 450$)*

DISCUSSION

The intravenous administration of glucan prior to challenge with *S. aureus* was effective in modifying morbidity and mortality due to systemic staphylococcal infection. The apparent loss of protection in the later stages of the disease may reflect a loss of protective efficacy due to the reversible nature of the glucan-induced hyperfunctional state (6, 10, 20). The current studies demonstrating the ability of glucan to modify *S. aureus* infection agree with previous findings that have shown protection in murine infections with *Sporotrichum schenckii* (M. Stevens, P. Steven, J. A. Cook, H. Ichinose, and N. R. Di Luzio, *J. Reticuloendothel. Soc. Prog. Abstr.* 66a, 1976), *Candida albicans* (D. L. Williams, J. A. Cook, E. O. Hoffmann, and N. R. Di Luzio, *RES J. Reticuloendothel. Soc.*, in press), *Cryptococcus neoformans*, and *Mycobacterium leprae* (F. Lejeune, J. Delville, J. Gillet, M. Song, S. Stadsbaeder, and P. Jacques, *Eur. J. Cancer*, in press). These studies with bacterial and fungal pathogens, both opportunistic and overt, denote that glucan induces a state of nonspecific host resistance.

The exact mechanism by which glucan prevents sequelae to disseminated staphylococcal disease has not been fully defined. However, earlier studies have demonstrated that glucan enhances both number and function of macrophage populations (6). Additionally, Burgaleta and Golde have reported enhanced leukopoiesis following glucan administration (5). They observed a twofold increase in peripheral leukocyte count along with an increase in the total macrophage content of the spleen, bone marrow, and peritoneal cavity of glucan-treated mice. Other parameters of the immune system, such as complement (C3) (M. Glovsky, N. Di Luzio, and L. Ghekiere, *J. Reticuloendothel. Soc. Prog. Abstr.*, 54a, 1976) and serum lysozyme (P. Kokoshis, D. L. Williams, J. A. Cook, and N. R. Di Luzio, *Science*, in press), have also been shown to increase due to glucan stimulation. This enhanced state of innate defenses, promoted by glucan and primarily mediated by phagocytes, may play an important, if not crucial, role in protection of the host against systemic staphylococcal disease.

Based on earlier studies, mouse peritoneal exudate macrophages have been shown to phagocytize and destroy non-encapsulated strains of *S. aureus* (2). Biggar et al. have reported similar data using rabbit alveolar macrophages against other gram-positive cocci, such as *Streptococcus faecalis* and *Streptococcus pneumoniae* (3). While other components of the immune system cannot be negated, the present observations, in conjunction with the above-cited studies, tend

to support the role of macrophages in host defense against *S. aureus* infections.

Studies with other immunostimulants, such as BCG and *Corynebacterium parvum*, have not shown an effect comparable to glucan on survival of mice with experimentally induced staphylococcal septicemia. Adlam et al. reported no consistent effect with intravenous or intraperitoneal administration of *C. parvum* in mice that were subsequently challenged intravenously with *S. aureus* (1). In contrast, the data presented here show that glucan provides significant protection with intravenous challenge doses of *S. aureus* up to an order of magnitude higher than the intraperitoneal challenge dose reported by Adlam et al. (1). Sher and co-workers have reported that intraperitoneal pretreatment with BCG will modify survival of mice challenged with 2.5×10^8 *S. aureus* (17). In comparison, our data demonstrated that intravenous administration of glucan promotes significant protection against intravenous challenge doses as high as 10^9 *S. aureus*. Whether the failure of *C. parvum* to modify *S. aureus* lethality is due to the differential toxicity of glucan and *C. parvum* (13) remains to be established.

The present data also demonstrated the protective efficacy of a glucan post-treatment regimen against syngenic murine lymphocytic leukemia. Of equal importance, however, glucan was effective in significantly modifying staphylococcal infection in leukemic mice. Secondary infections in leukemic children are of increasing clinical concern, and *S. aureus* is commonly implicated as one of the gram-positive organisms initiating secondary infections in the leukemic host (12). In view of these observations, prophylactic treatment with glucan may be of potential value in combating staphylococcal sepsis, particularly as secondary infections in malignant episodes.

Based upon histological examination, the kidneys appear to be the most vulnerable organ following the intravenous administration of *S. aureus*. This observation is in agreement with the data reported by Gorrill (8). Adlam et al. have suggested that the lack of protective efficacy of *C. parvum* administration against systemic *S. aureus* septicemia may be the result of the predilection of these microorganisms for the kidney (1). It was further postulated that the kidney is not affected by *C. parvum*-induced lymphoreticular stimulation, and thus the protective efficacy of *C. parvum* is lost following systemic infection with a nephrophilic microorganism. In contrast, our data suggest that glucan stimulation of the reticuloendothelial system is effective in preventing degenerative changes in

the kidney. The protective efficacy of glucan administration in *S. aureus*-treated mice is manifested by prolonged survival as well as minimal pathological changes in the kidneys of glucan animals as compared with controls.

Our observations also denote that glucan increases resistance to staphylococcal septicemia and may confer duality of therapy by modifying the course of systemic leukemia while simultaneously enhancing host resistance to *S. aureus* infection.

ACKNOWLEDGMENTS

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