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Passive Transfer of Poly-(1-6)-β-Glucotriosyl-(1-3)-β-Glucopyranose Glucan Protection against Lethal Infection in an Animal Model of Intra-Abdominal Sepsis

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Previous studies have established the efficacy of soluble polymers of poly-(1-6)-β-glucotriosyl-(1-3)-β-glucopyranose (PGG) glucan, a biological-response modifier, in protecting against mortality associated with experimentally induced peritonitis in a rat model. PGG glucan-treated animals showed increases in total leukocyte counts and enhanced bacterial clearance from blood. To further explore the mechanism(s) by which this agent confers protection, studies were performed to examine whether protection could be transferred from PGG glucan-treated animals to naive recipients via spleen cells (SC), SC lysates, or serum. Passive-transfer experiments indicated that the responsible factor(s) was transferable by whole SC and SC lysates, as well as by peripheral leukocytes or serum from animals treated with PGG glucan. The transferable factor(s) was resistant to pronase and trypsin digestion, was heat stable at 56 or 80°C, and was not removed by NH_4SO_4 precipitation. The protective effect of PGG glucan was abrogated by treatment with indomethacin, a potent inhibitor of prostaglandin synthesis. Administration of a purified prostaglandin extract from the sera of PGG glucan-treated animals protected against mortality in the peritonitis model. Furthermore, treatment of rats with exogenous synthetic prostaglandin E_2 also conferred protection against mortality. These results suggest that the protective effect exhibited by PGG glucan in the rat peritonitis model is mediated, at least in part, by prostaglandins.

The anti-infective effect of poly-(1-6)-β-glucotriosyl-(1-3)-β-glucopyranose (PGG) glucan (Betafectin; Alpha-Beta Technology, Worcester, Mass.) has been well documented in experimental animal model systems (10), as well as in phase II human clinical trials (1). The ability of the constituents of *Saccharomyces cerevisiae* cell walls to activate various components of the host immune response has been known since the early 1960s (15), although the more recent finding by Czop and Austen of a specific membrane receptor for β-glucans on human neutrophils and macrophages has provoked more intense interest in PGG glucan as a modulator of the host immune response (6). It has been shown that host defense responses related to the β-glucans include the activation of the alternative complement pathway, the release of lysosomal enzymes by monocytes, and the generation of leukotrienes by monocytes (6, 7). The experimental models of peritonitis employed in our studies have been used extensively for evaluating therapeutic regimens during an infectious process simulating intra-abdominal sepsis in humans (5, 9, 10). These models utilize intraperitoneal (i.p.) challenge with cecal contents (in the rat model) or monomicrobial challenge with *Escherichia coli* or *Staphylococcus aureus* (in the mouse model). In each model, the peritonitis phase of the experimental disease process is characterized by a mortality rate of 50% or greater and septicemia. The protective effect of PGG glucan for mice challenged i.p. with either *E. coli* or *S. aureus* has been demonstrated (10). Previously, we have shown that PGG glucan yielded protection against mortality associated with experimental intra-abdominal sepsis in mice and rats. In addition, animals treated with PGG glucan showed enhanced clearance of bacteria from blood and increased total leukocyte counts. These studies suggested that there is no direct antibacterial activity of the PGG glucan per se and that protection is based on modulation of the host response to infectious challenge (10).

To better understand the mechanism(s) of protection induced by PGG glucan, additional studies were performed using the passive transfer of cells and serum from treated animals to untreated recipients as a method for the characterization of this protective effect following infective challenge.

MATERIALS AND METHODS

Animals. Male Wistar virus-antibody-free rats (Charles River Breeding Labs, Wilmington, Mass.) weighing 176 to 200 g were utilized for all experiments. All animals were housed five per cage within a virus-antibody-free facility and were given food and water ad libitum, except for those animals used for the preparation of the meat-fed cecal inoculum (see below). Animal experiments were carried out according to the guidelines of the Harvard Medical Area Standing Committee on Animals, in facilities accredited by the American Association for the Accreditation of Laboratory Animal Care.

PGG glucan. A soluble polymer of PGG glucan, obtained from genetically engineered yeast cells and produced as an unmodified polysaccharide (Betafectin; Alpha-Beta Technology), was prepared in a sterile, pyrogen-free saline solution. The solution was administered by intramuscular injection 24 and 4 h prior to bleeding, spleen removal, or surgical implantation of the bacterial inoculum. A dosage of 100 μg per animal per injection was used for all experiments. Individual lots of material were assayed by the limulus amoebocyte lysate method (BioWhitaker, Walkersville, Md.) to ensure the absence of endotoxin.

Isolation of leukocytes from whole blood. Animals were placed under anesthesia (sodium pentobarbital, 50 mg/kg of body weight) and exsanguinated by percutaneous transthoracic cardiac puncture. The blood was mixed with an equal volume of a sterile heparin buffer composed of 2% dextran T500, 0.9% NaCl, and heparin (final concentration, 10 U/ml). This mixture was incubated for 1 h at 37°C to allow sedimentation. The top plasma layer was transferred to a centrifuge tube and spun at 2,000 × *g* for 20 min. The pelleted cells were washed twice in 20 ml of 0.9% saline and counted, and the cell density was adjusted with Eagle's minimal essential medium to a concentration of 1×10^7/ml for i.p. injection into recipient animals (2).

Preparation of spleen cells (SC). Animals were euthanized with CO_2, and their

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spleens were excised aseptically and placed in sterile petri dishes containing Hanks balanced salt solution (HBSS) at an ambient temperature. The spleens were minced with sterile scissors, and the cells were released by gentle disruption of the tissue through sterile stainless steel wire gauze by using the rubber plunger of a 5-ml syringe as a pestle (11). The cell preparation was filtered through sterile Dacron gauze to facilitate the removal of cellular debris and clumped cells and then assayed for total cell density. Appropriate dilutions were made with HBSS, and the cell suspension was injected i.p. (4 h prior to bacterial challenge) into recipient animals.

Separation of T and B cells from SC. B cells and macrophages were separated from T cells by using nylon wool columns as previously described (11). Cells were loaded onto columns at 37°C for 1 h and then eluted with HBSS at 37°C at a rate of 1 drop/s. T cells, which pass through the column, were collected, centrifuged, resuspended in HBSS, and counted so that appropriate dilutions could be prepared for i.p. injection into recipient animals. HBSS at 4°C was run through the column to effect the release of B cells and macrophages adhering to the nylon wool. Finally, this fraction (B cells and macrophages) was prepared for injection as described above. The purity of each cell preparation was assessed by fluorescence-activated cell sorter analysis and found to be >95%.

SC lysate (SCL) preparation. Lysates of SC preparations were made by flash freezing suspensions of whole SC in liquid nitrogen and then thawing them at 30°C. This cycle was repeated a total of three times. After the final thaw, the lysates were filtered through sterile Dacron gauze to remove cellular debris, resuspended in HBSS to appropriate equivalent concentrations of SC, and injected i.p. into recipient animals.

Pronase, trypsin, and chloroform-ether treatment of SCL. To 4 ml of SCL was added an excess of pronase B or trypsin (approximately 1 mg). The preparation was incubated at 37°C for 2 h (sufficient for enzymatic action and residual enzyme degradation) and then injected i.p. at equivalent SC concentrations. The SCL was extracted with a 1:1 (vol/vol) mixture of chloroform and ether and centrifuged at 10,000 × *g* for 30 min. The pellet was reextracted and dissolved in HBSS. This preparation was then tested in the animal model at equivalent SC concentrations.

Ammonium sulfate treatment of SCL. NH_4SO_4 was added to SCL to a final concentration of 75% (wt/vol). The protein precipitate was pelleted after centrifugation at 10,000 × *g*. The resultant supernate was removed, dialyzed to remove the NH_4SO_4, and then injected i.p. into recipient animals.

Indomethacin treatment of animals. A dosage of 0.075 mg twice a day per animal was administered by the intramuscular route for 4 days prior to SC harvest, initiation of PGG glucan therapy, or bacterial challenge.

Animal model for intra-abdominal sepsis. The rat model for intra-abdominal sepsis was employed (17). In this model, a mixed microbial inoculum obtained from the cecal contents of meat-fed rats is surgically implanted into the peritoneal cavities of anesthetized rats through anterior midline incision, with gelatin capsules being used to contain the inoculum. The inoculum is prepared in a manner that consistently results in a mortality rate of 50 to 75% in untreated control animals as determined by the method of Reed and Muench (14). The model simulates the infectious process that occurs in humans following accidental peritoneal soilage with intestinal contents. This experimental infection is characterized by an early peritonitis associated with a high mortality and the presence of facultative gram-negative organisms within the blood and peritoneal cavity (12).

In experiments designed to examine the role of prostaglandins in protection against sepsis, a challenge inoculum consisting of *E. coli* 502501 (a clinical isolate obtained from the culture collection at Channing Laboratory) mixed with 1% dextran sulfate and 10% barium sulfate was employed. This inoculum was titrated such that 5.17×10^7 CFU yielded 50 to 70% mortality in the animal model as previously described (10). An i.p. monomicrobial challenge with *E. coli* has been routinely used in our laboratory to simulate the mortality phase of sepsis and yields a disease process similar to that which is induced by challenge with cecal contents. Previous studies with PGG glucan demonstrated that this polysaccharide elicits comparable protection against cecal-contents challenge and *E. coli*-induced mortality (10). Numerous studies have utilized lethal challenge with *E. coli* to study the effects of prostaglandins in modulating gram-negative sepsis (4, 16) and precipitated our efforts to discern their role in PGG glucan-elicited protection in our model of sepsis. Animals were observed four times per day for the first 48 h and twice per day thereafter for a 7-day period. Visibly moribund animals were humanely sacrificed with CO_2.

Prostaglandin extraction from serum. Prostaglandins were extracted from pooled serum samples obtained from naive or PGG glucan-treated rats (five rats per group) and purified by using an octyldecyl silane-silica gel cartridge as described by Powell (13). This procedure is specific for the isolation of prostaglandins and thromboxanes. In brief, absolute ethanol was added to each sample to a final concentration of 15%; samples were then centrifuged at 375 × *g* for 10 min at 4°C, and supernatants were retained and treated with 3% formic acid to achieve pH 3.0. Octyldecyl silane-silica cartridges (10 ml, with a 500-mg binding capacity; Varian, Harbor City, Calif.) were sequentially washed with 10 ml of distilled water, 10 ml of ethanol, and 5 ml of water. Samples were then applied to the cartridges. The cartridges were washed with 5 ml of distilled water, 5 ml of 15% ethanol, and 5 ml of petroleum ether prior to elution of the prostaglandin fraction with 10 ml of ethyl acetate. The samples were dried under nitrogen gas at room temperature, and each resulting product was resuspended in physiological saline (pH 7.0). Determination of the quantity of prostaglandin E_2 (PGE_2) in this fraction was accomplished by using a commercially available molecule-specific enzyme-linked immunosorbent assay (ELISA) kit (Perseptive Diagnostics, Cambridge, Mass.). All samples were assayed in duplicate wells, and absorbances were determined by using an enzyme immunoassay plate reader (Biotek Instruments, Winooski, Vt.) at 405 nm. PGE_2 concentrations were calculated from standard curves generated with accompanying eicosanoid standards.

TABLE 1. Efficacy of serum or whole-cell transfer[a] in preventing mortality induced by cecal-contents challenge

Treatment	Mortality	*P* vs control
Saline	15/20 (75%)	NS[b]
PGG glucan	3/37 (8%)	<0.0001
1×10^7 leukocytes (from naive animals)	12/16 (75%)	NS
1×10^7 leukocytes (from treated animals)[c]	2/16 (13%)	<0.001
Serum, 1 ml (from naive animals)	8/15 (53%)	NS
Serum, 1 ml (from treated animals)[c]	1/16 (6%)	<0.01
1×10^7 saline-treated SC	10/15 (67%)	NS
1×10^6 activated SC[c]	1/21 (5%)	<0.0001
$\leq 4 \times 10^6$ activated T cells[c]	3/15 (20%)	<0.01
$\leq 4 \times 10^6$ activated B cells[c]	4/15 (27%)	<0.05

[a] Transfer was performed 4 h prior to implantation of inoculum.
[b] NS, not significant (control).
[c] From animals treated with PGG glucan 24 and 4 h prior to cell harvest.

Treatment with a purified prostaglandin extract or synthetic PGE_2. Rats were administered 1 ml of diluted, silica-purified prostaglandin extract obtained from PGG glucan- or saline-treated animals as described above. The purified preparation was diluted in sterile pyrogen-free saline and administered by the i.p. route 4 h prior to challenge with the *E. coli* monomicrobial-challenge inoculum. The amount of purified extract given was standardized to equal the amount of PGE_2 detected in 1 ml of serum from saline- or PGG glucan-treated animals as determined by the PGE_2-specific ELISA. Groups of animals were also treated with 1:5 or 1:10 dilutions of the purified preparation.

A commercially available, purified, synthetic PGE_2 preparation (Sigma Chemical Co., St. Louis, Mo.) was tested for its ability to elicit protection against lethal challenge in the animal model. Sterile PGE_2 was resuspended in absolute ethanol to a concentration of 10 mg/ml and further diluted with pyrogen-free saline (pH 7.0). Three doses of PGE_2 equivalent to those employed in the experiment described above were tested. PGE_2 was administered by the i.p. route 4 h prior to challenge with the *E. coli* challenge inoculum.

Statistical evaluation. Comparison of groups with regard to mortality was made by chi-square analysis as supplied on commercially available statistical software (InStat; GraphPad Software, Inc., San Diego, Calif.).

Experimental design. Earlier studies showed that PGG glucan enhanced bacterial clearance and raised total leukocyte counts in treated animals (10). Experiments were planned to further characterize the action of PGG glucan therapy. Passive transfers of serum, SC, T- and B-cell-enriched preparations, and SCL from animals treated with PGG glucan or with saline to untreated animals were performed. These transfers were carried out to determine whether protection could be passively transferred from viable cells or cell lysate preparations from animals actively treated with PGG glucan and, if so, by which cellular constituents such transfers were achieved. All transferred preparations were tested for the presence of PGG glucan to rule out the possibility that residual polysaccharide was responsible for protective activity. Further experiments involved the transfer of prostaglandin preparations that had been extracted from the sera of saline- or PGG glucan-treated animals and purified. Finally, synthetic PGE_2 was administered to animals to assess the ability of this molecule to reduce mortality associated with lethal challenge. All animal experiments included at least six animals per group.

RESULTS

Passive transfer of protection. The efficacy of PGG glucan treatment was first established in the animal model. Animals treated with PGG glucan were protected against mortality induced by cecal-contents challenge compared with saline-treated control animals (Table 1; $P < 0.0001$). Challenge with cecal contents yielded a 50 to 75% mortality rate in saline-treated animals. All animals that succumbed to the infection

TABLE 2. Efficacy of SCL transfer[a] in preventing mortality induced by cecal-contents challenge

Treatment	Mortality	*P* vs control
Lysate of 1×10^7 SC (naive)	22/33 (67%)	NS[b]
Lysate of 1×10^7 SC (activated)[c]	0/24 (0%)	<0.001
Lysate of 1×10^7 SC (activated)[c], 56°C, 30 min	0/6 (0%)	<0.01
Lysate of 1×10^7 SC (activated)[c], 80°C, 30 min	0/6 (0%)	<0.01
Lysate of 1×10^7 SC, pronase treated	1/6 (17%)	<0.05
Lysate of 1×10^7 SC, trypsin treated	0/6 (0%)	<0.01
Lysate of 1×10^7 SC, chloroform-ether extracted	3/6 (50%)	NS
Lysate of 2.5×10^6 T cells (activated)[c]	2/10 (20%)	<0.05
Lysate of 2.5×10^6 B cells (activated)[c]	1/9 (11%)	<0.01
Lysate of 1×10^7 SC (naive), NH_4SO_4 treated	4/6 (67%)	NS
Lysate of 1×10^7 SC (activated), NH_4SO_4 treated	0/6 (0%)	<0.05

[a] Transfer was performed 4 h prior to implantation of inoculum.
[b] NS, not significant versus control.
[c] From animals treated with PGG glucan 24 and 4 h prior to cell harvest.

did so within a 48-h period following challenge, with approximately 70% of the animals dying within the first 24 h.

To determine the source of the protective activity, passive-transfer experiments were performed in which serum, leukocytes, SC, or isolated lymphocyte populations from PGG glucan-treated animals were transferred to naive animals, which were subsequently challenged with the cecal-contents inoculum. The results of these experiments are shown in Table 1. The transfer of leukocytes from treated animals resulted in significant protection against mortality compared with transfer of cells from saline-treated animals. Serum transferred from PGG glucan-treated animals also conferred protection against infectious challenge compared with serum transferred from saline-treated rats ($P < 0.01$). Further experiments showed that the transfer of either whole SC or T- and B-cell-enriched populations from animals pretreated with PGG glucan significantly decreased the mortality associated with this experimental infection (Table 1).

Passive transfer of cell lysates. To further characterize the observed protection, lysates of SC preparations were injected into recipient animals to determine whether a specific subcellular factor(s) was responsible for the observed protection. Passive transfer of lysates prepared from SC or from T or B cells obtained from PGG glucan-treated animals afforded protection against lethal infection with cecal contents compared with SCL obtained from untreated controls (Table 2). In addition, it was observed that the factor responsible for this protection was not trypsin or pronase sensitive and was heat stable at 80°C for 30 min. The protective activity was abrogated in cell lysate preparations following chloroform-ether extraction, suggesting that a nonpolar, inflammation-modulating component might be associated with the protective effect. Furthermore, repeated freezing and thawing had little effect on the potency of this protective factor (data not shown).

Indomethacin treatment. In order to determine whether a component of the arachidonic acid pathway was involved in the protective action of PGG glucan during intra-abdominal sepsis, studies were done to evaluate the protective efficacy of SCL obtained from PGG glucan-treated animals following administration of the prostaglandin inhibitor indomethacin. The results (Table 3) indicate that indomethacin treatment of animals prior to PGG glucan administration decreases the protective effect of PGG glucan. This effect was observed in animals actively treated with PGG glucan prior to challenge with cecal contents or challenged following passive transfer of SCL from glucan-treated animals.

TABLE 3. Effect of pretreatment with indomethacin on PGG glucan-induced protection against mortality

Treatment	Mortality	*P*
Saline	3/19 (68%)	NS[a]
SCL (1×10^7 SC) from PGG glucan-treated animals[b]	0/8 (0%)	0.005[c]
PGG glucan[b]	0/19 (0%)	0.0001[c]
Indomethacin[d]	11/18 (61%)	<0.005[e]
SCL (1×10^7 SC) from indomethacin-treated animals[d]	12/18 (67%)	<0.005[e]
PGG glucan[b] plus indomethacin[d]	10/18 (56%)	<0.005[e]
SCL (1×10^7 SC) from animals treated with PGG glucan[b] and indomethacin[d]	10/18 (56%)	<0.005[e]

[a] NS, not significant (control).
[b] Animals treated with 100 μg of PGG glucan 24 and 4 h prior to bacterial challenge.
[c] *P* versus control.
[d] Animals treated with 0.15 mg of indomethacin 4 days prior to PGG glucan treatment or bacterial challenge.
[e] *P* versus PGG glucan treatment.

Protection by prostaglandin extracts from sera of PGG glucan-treated rats. The role of arachidonic acid metabolites in mediating the protective effect of PGG glucan was tested in the animal model. In these experiments, prostaglandins were purified from the sera of pyrogen-free saline- or PGG glucan-treated rats and were administered to naive recipients 4 h prior to challenge with *E. coli*. The purification procedure utilized is specific only for oxygenated metabolites of arachidonic acid and therefore is specific for prostaglandins (including PGE_2) and thromboxanes. Subsequently, the amounts of PGE_2 present in the purified preparations from control and experimental animals were determined by ELISA. Extracts purified from sera were administered to recipient animals in amounts equivalent to that present in 1 ml of serum from a single saline- or PGG glucan-treated animal (80 and 1,430 pg, respectively; data not shown). Animals also received a 1:5 or 1:10 dilution of the preparation purified from sera of PGG glucan-treated rats. Administration of the purified serum preparation to recipients yielded significant protection at the highest dose employed, reducing mortality from 80% in the control group to 10% in the experimental group (Table 4). Treatment of animals with a 1:5 dilution of this dose reduced mortality to 40% (not signif-

TABLE 4. Effect of PGG glucan-induced prostaglandin on mortality following challenge with *E. coli*

Prostaglandin concn (pg)[a]	Mortality	*P* vs control
80 (saline treated)	8/10 (80%)	NS[b]
1,430	1/10 (10%)	<0.005
286 (1:5 dilution)	4/10 (40%)	0.09
143 (1:10 dilution)	1/8 (11%)	<0.005

[a] Animals were administered serum-purified extract i.p., equivalent to the concentration of PGE_2 in 1 ml of serum taken from saline- or PGG glucan-treated rats (80 and 1,430 pg/ml, respectively) as determined by ELISA, 4 h prior to challenge.
[b] NS, not significant (control).

TABLE 5. Effect of synthetic PGE_2 on mortality following challenge with *E. coli*

Synthetic PGE_2 concn (pg)[a]	Mortality	*P* vs control[b]
0 (saline treated)	4/8 (50%)	NS
1,500	0/9 (0%)	<0.05
300	0/9 (0%)	<0.05
150	3/8 (38%)	NS

[a] Animals were administered PGE_2 i.p. 4 h prior to challenge.
[b] NS, not significant.

icant), while animals given a 1:10 dilution of the highest dose had an 11% mortality rate ($P < 0.005$).

Protection by exogenous PGE_2. To confirm the ability of PGE_2 to protect against *E. coli* challenge in the animal model, a commercially available synthetic preparation of this eicosanoid compound was tested. Animals received doses of synthetic PGE_2 comparable to the doses of silica-purified PGE_2 preparations used in the previous experiment and were challenged with *E. coli*. Animals treated with 1,500 or 300 pg of PGE_2 exhibited significant protection against mortality when compared with the saline-treated control group (Table 5; $P < 0.05$). However, a significant reduction in mortality rate was not seen in animals treated with the lowest dose tested (150 pg).

DISCUSSION

Previous studies have clearly shown that pretreatment of animals with PGG glucan prior to bacterial challenge results in significant protection against lethality in an animal model of intra-abdominal sepsis (10). In addition, PGG glucan-treated animals exhibited enhanced bacterial clearance and higher total leukocyte counts. These results have proven to be reproducible from experiment to experiment and with different preparations of PGG glucan. To further characterize the mechanism by which this carbohydrate confers protection against lethality, a series of passive-transfer experiments were performed.

The ability to passively transfer protection to untreated animals with SC from PGG glucan-treated animals suggested that a particular immune-cell type or cell-associated factor was responsible for the protective effect. The fact that protection can be passed with both T- and B-cell-enriched populations of SC and lysates of these cell populations further indicated that the effect of PGG glucan may not be specific for a particular cell subset. Experiments using cell lysates or serum from treated animals for passive transfer of protection suggested that a soluble effector is capable of reducing sepsis-associated mortality.

Studies to partially characterize this factor(s) indicated that it was not inactivated by heating at 56 or 80°C, was resistant to proteases, and did not precipitate with ammonium sulfate. Chloroform-ether extraction of SCL from PGG glucan-treated animals prior to passive transfer reduced the protective effect of the lysate compared with that of lysates from saline-treated animals, implying that there is a nonpolar lipid or fatty acid component associated with protection. The use of a prostaglandin synthesis inhibitor, indomethacin, reduced the efficacy of PGG glucan, further suggesting that the mediator of protection is a part of the arachidonic acid pathway. We have ruled out the possibility that biologically active PGG glucan present in the serum and cell preparations conferred protection in the animal model since the methods used to prepare the isolated B- and T-cell populations would exclude the presence of appreciable quantities of the glucan. Further, an organic solvent such as chloroform-ether does not solubilize PGG glucan but was capable of reducing the protective effect of SCL in the animal model.

The involvement of prostaglandins in mediating PGG glucan-induced protection against sepsis-associated mortality was assessed. Experiments were performed in which a purified prostaglandin preparation obtained from PGG glucan-treated animals transferred protection against lethal challenge. Animals receiving silica-purified prostaglandins from the PGG glucan-treated animals had a significantly lower mortality rate when compared with the control group. The implication of this compound's role in protection was tested in a subsequent experiment in which synthetically derived exogenous PGE_2 was administered to animals prior to lethal challenge with *E. coli*. The ability of PGE_2 to significantly reduce mortality in this case demonstrated that this compound most likely has an important role in the protective effect associated with PGG glucan treatment.

There is much debate concerning the participation of prostaglandins in inflammatory processes associated with trauma and disease (4, 8, 16). Although some studies indicate that prostaglandins have deleterious effects in animal models of endotoxemia (8), others have shown that PGE_2 has immunosuppressive effects in burn patients as well as in mouse models of sepsis (16). The last work demonstrated that the administration of a long-acting derivative of PGE improved survival in C3/HEN mice challenged with *E. coli*. The disparate biological effects noted with regard to the testing of prostaglandins could be readily attributable to many factors, including the route of administration, the dose, or the animal system employed.

Previous studies have demonstrated that polysaccharides from yeast cell walls release metabolites of the arachidonic acid pathway from host immune cells, such as alveolar macrophages and monocytes (3, 6). The work of Czop and Austen (6, 7) in particular has shown that leukotrienes, arachidonic acid metabolites of the lipooxygenase pathway, are released from human monocytes in response to β-glucans through interaction with a specific receptor on these cells. This work suggests that these molecules are important in controlling the immunomodulatory effects of PGG glucan and may provide a rationale for our findings. In our study, it is clear that prostaglandin levels are increased in PGG glucan-treated animals and can be extracted from the sera of treated animals to confer protection against mortality induced by lethal challenge. The ability of synthetically produced, exogenous PGE_2 to improve survival in infected animals also supports this contention. Taken together with our earlier work, these studies demonstrate that PGG glucan has multiple effects in protecting animals against bacterial sepsis. First, this compound enhances bacterial clearance and increases total leukocyte counts in treated animals. Second, we have now demonstrated that prostaglandins present in PGG glucan-treated animals also have a role in preventing mortality associated with sepsis. Further work is under way to determine the cellular mechanism by which PGG glucan exerts these biological effects.

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