INFECTION AND IMMUNITY, Apr. 1992, p. 1642–1647
0019-9567/92/041642-06$02.00/0

Vol. 60, No. 4

Anti-Infective Effect of Poly-β1-6-Glucotriosyl-β1-3-Glucopyranose Glucan In Vivo

ANDREW B. ONDERDONK,[1]* RONALD L. CISNEROS,[1] PAUL HINKSON,[1] AND GARY OSTROFF[2]

Department of Pathology, Channing Laboratory, Brigham and Women's Hospital, Harvard Medical School, 180 Longwood Avenue, Boston, Massachusetts 02115,[1] and Alpha Beta Technology, Worcester, Massachusetts 01605[2]

Received 12 November 1991/Accepted 22 January 1992

Mice challenged with *Escherichia coli* or *Staphylococcus aureus* were protected against lethal peritonitis by the intravenous administration of 10 μg of poly-β1-6-glucotriosyl-β1-3-glucopyranose (PGG) glucan per animal 4 to 6 h prior to bacterial challenge. Subsequent studies with the rat model for intra-abdominal sepsis indicated that intramuscular doses of 10 to 100 μg per animal 24 and 4 h prior to surgical implantation of the bacterial inoculum reduced the early mortality associated with the peritonitis phase of this experimental disease process. Quantitative cultures of blood obtained from challenged rats showed that significantly fewer organisms were present in the blood of PGG glucan-treated animals than in that of untreated animals. Quantitative studies of leukocytes of rats and mice following a single injection of PGG glucan showed a modest transient increase in the total leukocyte count. The possible mechanisms by which protection occurs in the animal model system are discussed.

The ability of yeast cell wall constituents to nonspecifically activate certain components of the host immune response has been known since the early 1960s, when a crude insoluble yeast cell wall extract, zymosan, was shown to be a stimulant of the reticuloendothelial system (23). Subsequent studies identified the active moiety of the yeast cell wall as β-glucan (1, 21). These insoluble glucose polymers alone or as vaccine adjuvants for viral and bacterial antigens markedly increase resistance to a variety of bacterial, mycotic, and viral infections (14, 22, 28). Studies have shown that β-glucan therapy increases the plasma and splenic levels of interleukins 1 and 2 in rats, an observation that, in part, may explain the broad-based anti-infective activity of these materials (8).

A recent finding by Czop and Austen (6) was the specific receptor binding site for β-glucan on the membrane of human neutrophils and macrophages. 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 (7, 9, 13).

Despite the exciting array of biologic activities that appear to be activated by the β-glucans, the fact that the material used in earlier studies was an insoluble extract of yeast cells rendered it less than desirable for clinical use, except as a vaccine adjuvant. Recent advances in the understanding of the composition and structure of the β-glucans have dramatically altered the possibilities for use of these materials as prophylactic and/or therapeutic immunomodulators. One of these β-glucans, poly-β1-6-glucotriosyl-β1-3-glucopyranose (PGG) glucan, obtained from genetically engineered yeast cells has been chemically well characterized and is produced as an unmodified polysaccharide (Betafectin; Alpha Beta Technology, Worcester, Mass.) (12, 19). The basic structure of this material is a β-D-(1-3)-linked glucopyranosyl backbone with periodic β-D-(1-6)-linked side chains (3, 24). PGG glucan is highly branched and has been shown to have a much higher affinity for the β-glucan receptor of the human monocyte than do other less-branched materials (2, 15, 16, 19, 25).

We present the results of in vivo mouse and rat studies designed to determine whether PGG glucan can abrogate the lethal process that occurs during acute intra-abdominal sepsis.

MATERIALS AND METHODS

Animals. Male CD-1 virus antibody-free (VAF) mice (Charles River Laboratories, Wilmington, Mass.) weighing 20 to 24 g each were used for basic characterization studies. Male VAF Wistar rats (Charles River Laboratories) weighing 180 to 200 g were used for studies employing the animal model for intra-abdominal sepsis. All animals were housed, five per cage, within a VAF facility and were given food and water ad libitum. Animal experiments were carried out according to the guidelines of the Harvard Medical Area Animal Use Committee.

Bacterial strains. *Staphylococcus aureus* (ATCC 29213) and *Escherichia coli* (TVDL 41, originally derived from rat cecal contents) were used as the challenge strains for studies with mice. Strains were grown in brain heart infusion broth supplemented with hemin and menidione (BHIS; Adams Scientific, Warwick, R.I.) for 24 h at 37°C and washed three times in phosphate-buffered saline (PBS, pH 7.0); aliquots were frozen at −80°C until used. Replicates of the frozen stock cultures were thawed at room temperature, serial decimal dilutions were made, and 0.1-ml samples of each dilution were plated onto Trypticase soy blood agar to determine the viable-cell density for the inoculum. All counts were reported as the $\log_{10}$ CFU per milliliter.

Cecal content inoculum. The inoculum used for rats as part of the model for intra-abdominal sepsis was obtained by removing the cecal contents from rats maintained on a diet of lean ground beef for a period of 2 weeks and diluting it with an equal volume of peptone-yeast-glucose broth and 10% (wt/vol) barium sulfate, as described in previous publications (18, 27). The bacteriologic characterization of this inoculum is that it is polymicrobic and includes an array of facultative

* Corresponding author.

TABLE 1. Composition of inoculum for intra-abdominal sepsis model in rats

Organism	Log_{10} CFU/animal
Escherichia coli	5.14
Proteus mirabilis	4.44
Streptococcus sp. group D	5.22
Streptococcus sp. non-group-A, -B, or -D	5.74
Streptococcus avium	4.44
Streptococcus faecalis	5.42
Bacteroides fragilis	6.96
Bacteroides sp.	5.26
Clostridium perfringens	6.56
Clostridium ramosum	7.24
Peptostreptococcus magnus	6.26
Peptostreptococcus productus	7.50
Unidentified gram-negative facultative anaerobe	6.26

species, such as *E. coli*, as well as obligate anaerobes (Table 1).

Quantitative cultures. Quantitative cultures of 0.1-ml samples of blood and peritoneal fluid were performed. Blood was obtained by percutaneous transthoracic cardiac puncture; peritoneal fluid was drawn through an anterior midline laparotomy incision from animals anesthetized with 0.25 ml of pentobarbital sodium (Nembutal; 50 mg/ml). Samples of blood were added directly to 20 ml of molten BHIS agar, mixed, and poured into sterile plastic petri plates. Once the agar had solidified, the plates were incubated at 37°C under anaerobic conditions for 24 to 48 h, and colonies were enumerated. Peritoneal fluid samples were diluted in sterile PBS to yield decimal dilutions from 10^{-2} to 10^{-7}, and aliquots were plated onto brucella-base blood plates. Following incubation, colonies were enumerated.

Bacterial challenge of mice. Mice were challenged with an approximately 70% lethal dose (LD_{70}) of either *S. aureus* or *E. coli* as determined by preliminary studies ($10^{7.7}$ CFU of *E. coli* per animal and $10^{7.65}$ CFU of *S. aureus* per animal). Challenge was performed by injection of 0.1 ml of bacterial suspension into the peritoneal cavity after dilution of the stock suspension to the appropriate concentration with sterile PBS. At the time of challenge, an aliquot of the challenge inoculum was used to verify the viable-cell density of the stock bacterial suspension. Animals were observed four times per day for the first 48 h and twice per day thereafter. Obviously moribund animals were humanely sacrificed with CO_2.

Animal model for intra-abdominal sepsis. The rat model for intra-abdominal sepsis has been well described in the scientific literature (18, 27). Briefly, a 0.5-ml aliquot of the thawed cecal content inoculum was placed into a gelatin capsule, and the capsule was surgically implanted into the peritoneal cavity of an anesthetized rat through an anterior midline incision. The incision was closed with 3-0 silk suture, and then the animals were returned to their cages. Animals were observed four times per day for the first 48 h and twice per day thereafter. Moribund animals were humanely sacrificed with CO_2.

Total and differential cell counts. For some experiments, peripheral blood samples were obtained by percutaneous, transthoracic cardiac puncture for assessment of total and differential counts of leukocytes (WBC). A 0.1-ml aliquot of blood was obtained after animals were lightly anesthetized with ether. The heparinized sample was placed into standard saline diluent, and the erythrocytes were lysed for total WBC determinations, using a Coulter model FN Counter (Coulter Electronics, Inc., Hialeah, Fla.). Smears of each sample were stained with Wright Giemsa stain, and the percentages of granulocytes, lymphocytes, and mononuclear cells were determined. The percentage of each cell type was then used to calculate the absolute cell counts by multiplying the percentage by the total cell count for each sample.

PGG glucan administration. PGG glucan (Betafectin) was used for all experiments. Stock PGG glucan, supplied as a soluble material at a concentration of 1.5 to 10.0 mg/ml, was prepared according to "Good Manufacturing Practices" (GMP) standards. Each lot used in animals was assayed by the limulus amoebocyte lysate (Associates of Cape Cod, Woods Hole, Mass.) method for the presence of endotoxin. Endotoxin levels were below the detectable limits of the assay (<0.03 endotoxin units per ml) in all materials used in these studies. The stock material was diluted in sterile, pyrogen-free saline solution for injection into rats and mice. Preliminary studies of anti-infective effects in mice indicated that the intravenous (i.v.) administration of PGG glucan by the percutaneous, transthoracic cardiac puncture method 4 to 6 h prior to challenge yielded protective results. Subsequent studies with rats indicated that two doses of 10 to 100 μg of PGG glucan per animal given intramuscularly (i.m.) at 24 and 4 h prior to challenge resulted in protection against lethal bacterial challenge. Except when stated otherwise, these routes and times of administration were used for all experiments.

Experimental design. Mice were used to evaluate the possible protective effect of PGG glucan in a peritoneal infection test system using *S. aureus* and *E. coli* at lethal concentrations. Neither animal test system was designed to simulate human disease but rather provided an assay system in which dose, response, time of administration, and hematologic changes could be evaluated before the more definitive intra-abdominal sepsis system was employed.

The rat model for intra-abdominal sepsis is a well-documented animal model that simulates an infectious process that occurs in humans following accidental peritoneal soilage with intestinal contents (18, 27). This infection is a two-stage process in which an early peritonitis associated with a high mortality and the presence of facultative gram-negative organisms within the blood and peritoneal cavity is followed by a more chronic process involving the formation of intra-abdominal abscesses, during which anaerobes have been shown to be important constituents (27). On the basis of preliminary dose and timing studies in mice, this model was

TABLE 2. Effects of various doses of PGG on *E. coli*-induced mortality in mice

Treatment group[a]	Mortality (%)	*P* value vs control[b]
Saline	24/29 (83)	
PGG (μg/animal)		
1,000	7/10 (70)	0.7
100	4/9 (44)	0.06
50	2/5 (40)	0.13
10	0/5 (0)	<0.05
1	2/9 (22)	<0.05
0.5	4/9 (44)	0.06

[a] All doses were given by percutaneous transthoracic cardiac puncture 4 h prior to challenge.

[b] Saline-treated control animals; chi-square analysis using Yate's correction.

TABLE 3. Effects of various doses of PGG on *S. aureus*-induced mortality in mice

Treatment group[a]	Mortality (%)	*P* value vs control[b]
Saline	5/8 (63)	
PGG (μg/animal)		
100	6/9 (67)	1.00
10	5/10 (50)	0.66
1	1/9 (11)	0.05

[a] All doses were given by percutaneous transthoracic cardiac puncture 4 h prior to challenge.
[b] Saline-treated control animals; chi-square analysis, Fisher exact test.

TABLE 5. Effects of PGG on mortality in a rat model for intra-abdominal sepsis

Expt	Group[a]	Mortality (%)	*P* value vs untreated
A	Untreated	5/10 (50)	
	PGG	3/30 (10)	0.02
B	Untreated	6/10 (60)	
	PGG	2/20 (10)	0.01

[a] Two doses of 100 μg of PGG per animal given i.m. at 24 and 4 h prior to surgical challenge.

used to further characterize dose-response information, route and time of administration, and peripheral cell response following PGG glucan therapy.

Statistical evaluation. Comparison of groups with regard to mortality was made by chi-square analysis; comparisons of quantitative data were made with Student's *t* test, as supplied on commercially available statistical software (Statistix, NH Analytical Software, Ann Arbor, Mich.).

RESULTS

Prevention of *E. coli*-induced mortality in mice. Preliminary experiments with an insoluble PGG glucan (Adjuvax; Alpha Beta Technology) at doses as low as 100 μg per mouse suggested that mortality in mice challenged intraperitoneally with *E. coli* could be abrogated by pretreatment with PGG glucan. In order to determine whether a soluble PGG glucan preparation (Betafectin) was also capable of this protective effect, groups of mice were challenged with *E. coli* following percutaneous, transthoracic administration of soluble PGG glucan at doses ranging from 0.5 to 1,000 μg per animal (Table 2). As can be seen, there is a dose-response relationship between the amount of soluble PGG glucan administered to these animals and the degree of protection afforded. There appears to be an active range for this protective effect (17), with doses either above or below this range not providing any protection in this animal test system. Depending on the route and time of administration, the active range is between 100 and 0.5 μg per animal.

Prevention of *S. aureus*-induced mortality in mice. In order to determine whether the protection against *E. coli*-induced mortality was specific only for facultative gram-negative rods, groups of mice were challenged with an $\simeq LD_{70}$ of *S. aureus* following treatment with soluble PGG glucan at doses ranging from 1 to 100 μg per animal (Table 3). Protection against the lethal challenge was provided by a dose of 1 μg per animal in this test system but not by higher doses of soluble PGG glucan.

Effect of soluble PGG glucan on peripheral WBC counts in mice. Further characterization of the in vivo response of mice following administration of PGG glucan was achieved by obtaining peripheral blood samples from animals following a single dose of 10 μg of PGG glucan per animal (Table 4). Total WBC counts appear to peak between 24 and 48 h after a single i.v. dose of PGG glucan. The percentage increase in the numbers of monocytes and granulocytes is greater than anticipated from the increase in total counts relative to those for saline-treated animals. Total and differential counts returned to approximately pretreatment levels within 72 h of a single dose of PGG glucan (17).

Effect of soluble PGG glucan on mortality in the rat model for intra-abdominal sepsis. The preliminary mouse studies provided important dose and route of administration information for subsequent experiments in an animal model system simulating intra-abdominal sepsis. Because the use of percutaneous, transthoracic cardiac puncture for i.v. administration is known to increase mortality during this experimental infection, total and differential WBC counts following i.m. and i.v. injection were compared to determine whether i.m. injection was an alternative method for administration of soluble PGG glucan. No differences between these two methods of administration were noted for total and differential cell counts (17); thus, administration of soluble PGG glucan for all experiments involving rats was by the i.m. route.

The results of representative experiments with similar PGG glucan preparations and the same bacterial inoculum are shown in Table 5. As can be seen, the administration of two doses of PGG glucan at 24 and 4 h prior to challenge significantly reduces the mortality in this model system compared with that for untreated controls. Because the mortality during this experimental infection is known to be due to facultative gram-negative rods and is associated with relatively large numbers of organisms in the blood and peritoneal cavity, quantitative bacteriologic studies were performed to determine whether there were any differences between soluble PGG glucan-treated animals and untreated

TABLE 4. Effects of PGG treatment on peripheral WBC counts in mice

Treatment	24 h after treatment			48 h after treatment		
	Total WBC	Granulocytes[a]	Monocytes[a]	Total WBC	Granulocytes	Monocytes
Saline	8,129 ± 1,294[b]	1,430 ± 233	604 ± 117	6,310 ± 1,926	1,522 ± 83	389 ± 120
PGG glucan[c]	13,220 ± 60[d]	2,919 ± 284[d]	2,381 ± 577[d]	10,990 ± 1,013[d]	2,979 ± 414[d]	2,010 ± 266
% Increase vs saline	63	104	294	74	96	417

[a] Absolute cell counts.
[b] Means ± standard errors; three or more animals sampled per group.
[c] Ten micrograms.
[d] $P < 0.05$; two-sample *t* test versus saline controls.

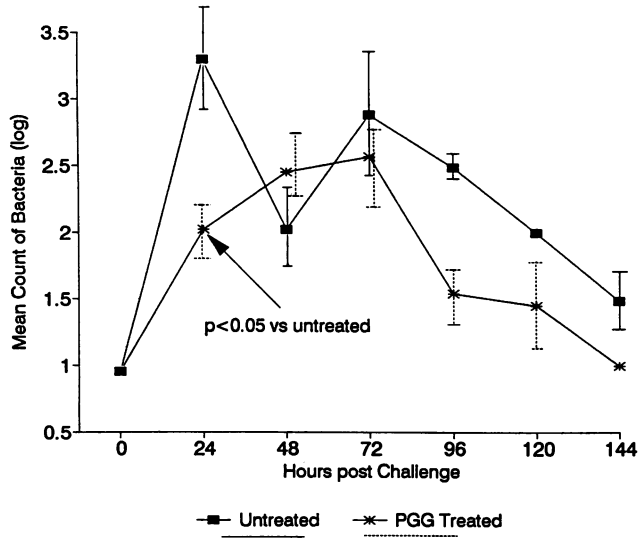

FIG. 1. Comparison of quantitative blood cultures obtained from rats following development of intra-abdominal sepsis. Two doses of 100 μg per animal were given i.m. at 24 and 4 h prior to challenge. Counts are expressed as the mean $\log_{10}$ CFU per milliliter for groups of two to nine samples per time point. A large initial group of animals was used for blood culture data because of deaths that were expected to occur over the course of the experiment. Moribund animals were sacrificed humanely and were not used as a source of blood culture data. Comparison of counts at 24 h postchallenge yielded a P value of <0.05 for PGG-treated animals (*) compared with untreated control animals (■), using a two-sample t test.

controls. The results of quantitative blood cultures (Fig. 1) indicate that there is an approximate 20-fold difference in the numbers of organisms per milliliter, with the soluble PGG glucan-treated animals having significantly fewer organisms present at 24 h, a time at which most deaths occur in this model system. A continued decrease in the number of organisms present occurs in the surviving recipients of the cecal challenge over the next several days, with the soluble PGG glucan-treated animals appearing to clear organisms from the blood more quickly than untreated animals. Additional studies have yielded similar quantitative data for treatment and control groups.

Quantitative peritoneal cultures. The numbers of organisms present in free-flowing peritoneal exudates from animals treated with soluble PGG glucan and from untreated animals were determined at various times after challenge. Within 96 h, the number of organisms measured in peritoneal exudates from treated animals was significantly lower ($10^{2.46}$ CFU/ml) than that noted for untreated animals ($10^{4.85}$ CFU/ml). Within 5 days of challenge, discreet intra-abdominal abscesses had formed in most animals and peritoneal exudates had decreased in quantity (Table 6).

An assessment of the total peripheral WBC count following administration of soluble PGG glucan yielded results similar to those for mice, with total WBC counts increasing almost 100% within 5 days in treated animals compared with those in untreated animals (Table 7).

TABLE 6. Comparison of quantitative peritoneal fluid cultures in a rat model for intra-abdominal sepsis

Time (h) post-challenge	Log_{10}CFU/ml (mean ± SE)[a]	
	Untreated	PGG treated[b]
24	4.83 ± 0.75	531 ± 0.16
48	5.66 ± 0.09	4.79 ± 0.68
96	4.85 ± 2.08	2.46 ± 0.76[c]
120	2.83 ± 0.42	1.16 ± 0.16[c]
144	3.05 ± 0.25	<1.0

[a] Two to five samples per group.
[b] Two doses of 100 μg per animal given i.m. at 24 and 4 h prior to challenge.
[c] $P < 0.05$, two-sample t-test.

DISCUSSION

Previous studies have documented the nonspecific immunomodulatory properties of insoluble polysaccharides, such as zymosan (4, 14, 22, 23, 28), in animal test systems. The earlier work with yeast glucans in animals involved a variety of uncharacterized, insoluble materials. Although it was clearly shown that such materials served as excellent adjuvants and as immunostimulants in their own right, the methods for administering such materials limited their usefulness to the laboratory.

The observation that yeast glucans could abrogate the deleterious effects of a variety of microbial pathogens prompted considerable interest in determining exactly how such compounds interact with the host immune system. The finding of a specific cell surface receptor for β-1,3 glucans on macrophages and granulocytes suggested that the protective effects for the glucans are provided by such cells (5, 6, 10, 26), possibly by cytokine production and release, improved phagocytic killing, or increased production of phagocytic cells. Because PGG glucans bind to cells with an array of immunomodulatory activities, it is likely that the actual mechanism(s) of protection provoked by such materials are complex.

Additional research has found soluble glucan polymers that appear to act in much the same manner as their insoluble parent compounds (9) and that might be used either therapeutically or prophylactically in humans at risk for microbial disease. Our research with mice was aimed at two essential tasks: (i) to find an in vivo test system to determine whether various preparations of soluble PGG glucans were biologically active in preventing lethal bacterial infections and (ii) to explore the basic host response following administration of soluble preparations by a variety of routes, using various doses and times of administration. Initial experiments in this laboratory revealed the protective effects of both soluble and insoluble parent materials in mice challenged with *E. coli*, effects that extend to gram-positive organisms on the basis of our experience with *S. aureus*. It should be noted that the protective effect was not related to endotoxin-induced tolerance, since none of the PGG glucan compounds contained measurable endotoxin. With either soluble or insoluble compounds there appeared to be an optimal dose range. Doses that were either higher or lower than this optimal range did not protect as well against the lethal challenge. When the numbers and types of WBC were evaluated after administration of soluble PGG glucan, a transient increase in the total cell counts and a corresponding increase in both granulocytes and monocytes were observed. Although not addressed in this study, i.v.-administered soluble PGG glucan at doses as high as 5 mg per animal was not toxic to mice.

Because the animal test system using mice did not represent a true animal model for an infectious process, we elected to continue the in vivo characterization of soluble

TABLE 7. Effects of PGG treatment on peripheral WBC counts in rats

Treatment	Day 1			Day 5		
	Total WBC	Granulocytes[a]	Monocytes[a]	Total WBC	Granulocytes	Monocytes
Untreated	4,035 ± 1,035[b]	1,814 ± 518	94 ± 31	3,307 ± 463	1,021 ± 116	131 ± 18
PGG[c]	5,067 ± 1,528	1,465 ± 1,436	527	12,260 ± 2,452[d]	5,883 ± 588[d]	808 ± 220

[a] Absolute cell counts.
[b] Means ± standard errors; minimum of two observations per sample time.
[c] One hundred micrograms.
[d] $P < 0.05$; two-sample t test versus untreated animals.

PGG glucan in the rat model for intra-abdominal sepsis. This model has been well characterized microbiologically (18, 27), and implantation of the cecal content inoculum routinely results in an ≈50% mortality, with all animals exhibiting a gram-negative septicemia during the first several days following challenge. It has also been shown that death in these animals is abrogated by antimicrobial therapy directed against facultative gram-negative rods, such as *E. coli*. Initial trials with soluble PGG glucan in this model system indicated that a reproducible reduction in mortality could be observed when soluble PGG glucan was administered i.m. in two doses at 24 and 4 h prior to challenge.

When tested at various times after challenge, soluble PGG glucan-treated animals had significantly fewer bacteria in their blood than did untreated animals, with a difference of at least 20-fold noted at 24 h postchallenge, a time when most deaths occur in infected animals. Peripheral WBC counts in rats given a single dose of soluble PGG glucan yielded the same proportional increases in total numbers of WBC as were noted in mice.

There is some evidence that soluble PGG glucan treatment primes granulocytes and macrophages for subsequent cytokine release (interleukin-1 and -2) when bacterial challenge occurs (26) and that soluble PGG glucan treatment acts in a manner similar to treatment with colony-stimulating factors (20). However, the timing of administration makes it unlikely that cytokines are the exclusive mechanism by which protection occurs. It is also clear that the differences in the number of bacteria present in blood cannot be explained simply by the presence of more phagocytic cells in the peripheral circulation.

On the basis of the observations regarding the total WBC counts and the number of organisms in the blood of treated animals, there are at least two additional possible explanations for the activity of soluble PGG glucan: (i) phagocytic killing is much more efficient following soluble PGG glucan treatment or (ii) bacteria do not leave the peritoneal cavity and enter the blood as effectively in soluble PGG glucan-treated animals because of improved phagocytic killing within the peritoneal cavity or some other mechanism. Although there are reports of increased phagocytic killing of both bacteria and yeast cells by monocytes and neutrophils after soluble PGG treatment, it is difficult to accept a 20- to 50-fold-greater killing efficiency of WBC as the entire explanation for the biologic activity of this material (11). We are currently addressing the possibility that *E. coli* simply does not enter the blood as effectively in animals treated with soluble PGG glucan as it does in untreated control animals. Preliminary data suggest that trapping of organisms within the peritoneal cavity is an important part of PGG glucan-induced protection.

Irrespective of the ultimate explanation for the in vivo activity of soluble PGG glucan, this apparently nontoxic, glucose polymer is an interesting immunomodulatory drug. If the activity noted in both animal test systems and the animal model system for intra-abdominal sepsis applies to larger animals, including humans, it is possible that such materials may either be used adjunctively with existing therapeutic modalities or replace less-appealing therapeutic methods of preventing and treating serious bacterial sepsis.

REFERENCES

1. **Bacon, J. S. D., V. C. Farmer, D. Jones, and I. F. Taylor.** 1969. The glucan component of the cell wall of Baker's yeast (*Saccharomyces cerevisiae*) considered in relation to its ultrastructure. Biochem. J. **114**:556–557.
2. **Bluhm, T., Y. Deslandes, and R. Marchessault.** 1982. Solid-state and solution conformation of scleroglucan. Carbohydr. Res. **100**:117–130.
3. **Bluhm, T. L., and A. Sarko.** 1977. The triple helical structure of lentinan, a linear β-1-3-D-glucan. Can. J. Chem. **55**:293–299.
4. **Cook, J. A., T. W. Holbrook, and W. J. Dougherty.** 1982. Protective effect of glucan against visceral leishmaniasis in hampsters. Infect. Immun. **37**:1261–1269.
5. **Czop, J. K.** 1986. Phagocytosis of particulate activators of the alternative complement pathway: effects of fibronectin. Adv. Immunol. **38**:361–398.
6. **Czop, J. K., and K. F. Austen.** 1985. A β-glucan inhibitable receptor on human monocytes: its identity with the phagocytic receptor for particulate activators of the alternative complement pathway. J. Immunol. **134**:2588–2593.
7. **Czop, J. K., and K. F. Austen.** 1985. Generation of leukotrienes by human monocytes upon stimulation of their β-glucan receptor during phagocytosis. Proc. Natl. Acad. Sci. USA **82**:2751–2755.
8. **Czop, J. K., and K. F. Austen.** 1985. Properties of glycans that activate the human alternative complement pathway and interact with the human monocyte β-glucan receptor. J. Immunol. **135**:3388–3393.
9. **Czop, J. K., D. T. Fearson, and K. F. Austen.** 1978. Opsonin-independent phagocytosis of activators of the alternative complement pathway by human monocytes. J. Immunol. **120**:1132–1138.
10. **Easson, D. D., Jr., G. R. Ostroff, and S. James.** 1990. Macrophage-targeted carbohydrate microcapsules for antigen and drug delivery. Abstr. Int. Congr. Infect. Dis. 1990, abstr. 689, p. 58.
11. **Jamas, S., D. D. Easson, Jr., and G. R. Ostroff.** 1990. PGG—a novel class of macrophage activating immunomodulators. Abstr. Int. Congr. Infect. Dis. 1990, abstr. 698, p. 143.
12. **Jamas, S., D. D. Easson, Jr., G. R. Ostroff, and A. B. Onderdonk.** Submitted for publication.
13. **Janusz, M. J., K. F. Austen, and J. K. Czop.** 1987. Lysosomal enzyme release from human monocytes by particulate activators is mediated by β-glucan inhibitable receptors. J. Immunol. **138**:3897–3901.
14. **Kokoshis, P. L., D. L. Williams, J. A. Cook, and N. R. DiLuzio.** 1978. Increased resistance to *Staphyococcus aureus* infection and enhancement of serum lysozyme activity by glucan. Science **199**:1340–1342.
15. **Norisuye, T., T. Yanaki, and H. Fujita.** 1980. Triple helix of a

schizophyllum commune polysaccharide in aquaeous solution. J. Polym. Sci. Phys. Ed. **188**:547–558.
16. **Ogawa, K., and T. Watanabe.** 1973. The dependence of the conformation of a (1→3)-β-D-glucan on chain-length in alkaline solution. Carbohydr. Res. **29**:397–403.
17. **Onderdonk, A. B., R. L. Cisneros, P. Hinkson, and G. R. Ostroff.** Unpublished data.
18. **Onderdonk, A. B., W. M. Weinstein, N. M. Sullivan, J. G. Bartlett, and S. L. Gorbach.** 1974. Experimental intra-abdominal abscesses in rats: quantitative bacteriology of infected animals. Infect. Immun. **10**:1256–1259.
19. **Ostroff, G. R., D. D. Easson, Jr., and S. Jamas.** 1989. Manipulation of yeast glucan structure: molecular weight, branch frequency and branch length. 198th Am. Chem. Soc. Natl. Meet. 1989, abstr. MBTD O19.
20. **Patchen, M. L., and T. J. Macvittie.** 1986. Hemopoietic effects of intravenous soluble glucan administration. J. Immunopharmacol. **8**:407–425.
21. **Phaff, H. J.** 1963. Cell wall of yeasts. Annu. Rev. Microbiol. **17**:15.
22. **Reynolds, J. A., M. D. Kastello, D. G. Harrington, C. L. Crebs, C. J. Peters, J. V. Jemski, G. H. Scott, and N. R. DiLuzio.** 1980. Glucan-induced enhancement of host resistance to selected infectious disease. Infect. Immun. **30**:51–57.
23. **Riggi, S. J., and N. R. DiLuzio.** 1961. Identification of a reticuloendothelial stimulating agent in zymosan. Am. J. Physiol. **200**:297–300.
24. **Saito, H., T. Ohki, and T. Sasaki.** 1977. A 13C nuclear magnetic resonance study of gel-forming (1→3)-β-D-glucans. Evidence of the presence of single-helical conformation in a resilient gel of a curdlan-type polysaccharide 13140. Biochemistry **16**:908–914.
25. **Saito, H., T. Ohki, N. Takasuka, and T. Susaki.** 1977. A 13C-NMR-spectral study of a gel-forming, branched (1→3)-β-D-glucan (lentinan) from *Lentinus edodes*, and its acid-degraded fractions, structure, and dependence of conformation on the molecular weight. Carbohydr. Res. **58**:293–305.
26. **Sherwood, E. R., D. L. Williams, R. B. McNamee, E. L. Jones, I. W. Browdwe, and N. R. DiLuzio.** 1987. Enhancement of interleukin-1 and interleukin-2 production of soluble glucan. Int. J. Immunopharmacol. **9**:261–267.
27. **Weinstein, W. M., A. B. Onderdonk, J. G. Bartlett, and S. L. Gorbach.** 1974. Experimental intra-abdominal abscesses in rats: development of experimental model. Infect. Immun. **10**:1250–1255.
28. **Williams, D. L., J. A. Cook, F. O. Hoffman, and N. R. DiLuzio.** 1978. Protective effects of glucan in experimentally induced candidiasis. J. Reticuloendothel. Soc. **23**:479–490.