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Glucans and Cancer: Comparison of Commercially Available β-glucans – Part IV

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Abstract. *Background/Aim: β-Glucans are well-established immunomodulators with strong effects across all immune reactions. Due to the extensive amount of studies, glucans are steadily progressing from a non-specific immunomodulator to a licensed drug. However, direct comparisons of higher numbers of different glucans are rare. Materials and Methods: In this study, we used 16 different glucans isolated from yeasts, mushroom, algae, and oat and compared their effects on phagocytosis, IL-2 production, antibody secretion, and inhibition of three experimental cancer models. Results: Our results showed significant differences among tested glucans, showing that despite the fact that glucans in general have strong stimulating effects on most aspects of the immune system, it is necessary to choose the right glucan. Conclusion: Based on our studies, we can conclude that highly purified and active glucans have significant pleiotropic effects.*

β-1,3-D-glucans (hereafter referred to as "glucans") form part of a group of natural biologically-active compounds generally called "biological response modifiers." Chemically, they are heterogeneous nonstarch polysaccharides, which form structural compounds of the cell wall of some microorganisms including yeast, algae, protists, mushrooms, and grain. Generally, the term glucan is used as a chemical name of glucose polymer and represents a group of chemically heterogeneous carbohydrates. Typically, β-glucans form a linear backbone with 1-3 β-glycosidic bonds but vary with respect to molecular mass, solubility, viscosity, branching structure, and gelation properties. Insoluble β-glucans fibers consist of β-(1,3/1,4)-D-linked glucose units, whereas the soluble viscous fiber consist of β-(1,3)/1,6)-D-linked glucose.

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After more than 45 years of intensive research, biological effects of glucans are well-established. Various studies definitively show that glucans represent important substances exerting strong immunomodulatory properties and may be included, among other substances, to act through an organism's own biological response mechanisms (1). Among the well-studied effects of β-glucans, we can mention stimulation of both humoral and cellular immunity (1), metabolic control of diabetes (2), stimulation of wound healing (3), stress reduction (4), attenuation of chronic fatigue syndrome (5), lowering cholesterol levels (6), and inhibition of cancer (7). Recently, glucan was successfully used as part of a vaccine for high-risk neuroblastoma (8). In addition, a series of clinical studies showed strong effects on the treatment of children with chronic respiratory problems (9, 10). In Japan, glucan has been widely used, for over 30 years, in the treatment of gastrointestinal cancer (11).

Despite over 20,000 scientific studies, the results are still not fully compatible, with many individual glucans differing in characteristics, such as molecular weight, branching, solubility, and source, it is difficult to unify the described effects. The problem of diverse data can be solved only by comparative studies. However, scientific reports directly comparing individual glucans are still limited (12-16). This led us to the current comparative review of 16 different commercially available glucans.

Materials and Methods

Animals. Female, 8-week-old BALB/c mice were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). All animal work was done according to the University of Louisville IACUC protocol. Animals were sacrificed by CO_2 asphyxiation followed by cervical dislocation.

Materials. All glucans were either donated or purchased from the manufacturers or distributors as shown in Table I. Ovalbumin, polymyxin B, cyclophosphamide, and Wright stain were purchase from Sigma (St. Louis, MO, USA).

Cell lines. Murine melanoma B16 cell line (ATCC, Manassas, VA, USA), Lewis lung carcinoma cells (obtained from Dr. G. Ross, University of Louisville, Louisville, KY, USA), and BALB/c

Table I. *List of glucans used in this study.*

	Glucan	Source	Solubility	Manufacture
1	Beta Glucan 500 mg	Yeast	Insoluble	Priority One Vitamins
2	Beta Glucan (1,3/1,6) -Hypoallergenic	Yeast	Insoluble	Kirkman
3	Supreme Beta Glucan 95% 500 mg	Yeast	Insoluble	Hippo Herbs
4	Beta 1,3 Glucan #710	Yeast	Insoluble	Dee Cee Lab
5	ImmunotiX 500	Yeast	Insoluble	Xymogen
6	Beta Glucan,	Yeast	Insoluble	Wonder Laboratories
7	ImmunoMed 3-6	Yeast	Insoluble	NuMedica
8	Super Pure Beta 1,3 Glucan Algae Extract	Algae	Insoluble	The Synergy Company
9	Beta 1,3 Glucans	Yeast	Semi-Soluble	Puritan's Pride
10	Immune Support with βGlucan	Yeast, Mushrooms	Insoluble	Lindberg
11	Glucan Elite	Yeast	Semi-Soluble	Pro Formulations MD
12	Beta Glucan	Yeast	Semi-Soluble	Professional Formulas
13	Avena Sativa (Oat) Powder	Oat	Semi-Soluble	Maple Lifesciences
14	Beta Glucan 1,3-1,6	Yeast	Soluble	Bulk Supplements.com
15	Beta 1,3 Glucan	Yeast	Insoluble	AFI (America's Finest)
16	Glucan #300	Yeast	Insoluble	Transfer Point

mouse-derived mammary tumor cell line Ptas64 (generously provided by Dr. Wei-Zen Wei of the Michigan Cancer Foundation, Wayne State University, Detroit, MI, USA) were maintained in culture at 37˚C in a humidified atmosphere supplemented with 5% CO_2 in RPMI 1640 medium supplemented with 10% FCS.

Phagocytosis. Phagocytosis of synthetic polymeric microspheres was described previously (17). Briefly, 0.1 ml of peripheral blood from mice injected with various doses of glucan or PBS was incubated *in vitro* with 0.05 ml of 2-hydroxyethyl methacrylate particles (HEMA; 5×10^8/ml). The tubes were incubated at 37˚C for 60 min, with intermittent shaking. Smears were stained with Wright stain. The cells with three or more HEMA particles were considered positive. Mice were injected with either glucan or PBS (control). All experiments were performed in triplicate. At least 300 cells were examined in each experiment.

IL-2 secretion. Purified spleen cells (2×10^6/ml in RPMI 1640 medium with 5% FCS) obtained from mice injected with 100 μg glucan or PBS were added into wells of a 24-well tissue culture plate. Cells were incubated for 48 h in a humidified incubator (37˚C, 5% CO_2/95% air). Addition of 1 μg of Concanavalin A (Sigma, St. Louis, MO, USA) was used as a positive control. At the endpoint of incubation, supernatants were collected, filtered through 0.45 μm filters, and tested for the presence of IL-2 using a Quantikine mouse IL-2 kit (R&D Systems, Minneapolis, MN, USA).

Antibody formation. The technique was described earlier (18). Briefly, formation of antibodies was evaluated using ovalbumin as an antigen. Mice were injected twice (two weeks apart) with 100 μg of albumin and the serum was collected 7 days after last injection. Experimental groups received daily ip. injections of glucan. The level of specific antibodies against ovalbumin was detected by ELISA. As positive control, combination of ovalbumin and Freund's adjuvant (Sigma) was used.

Tumor inhibition in vivo. Mice were injected directly into their mammary fat pads with 1×10^6/mouse of Ptas64 cells in PBS. The experimental treatment was begun after palpable tumors were found (approximately 14 days after injection of cells) and after mice were assigned to experimental groups. Experimental treatment was achieved by intraperitoneal injections of tested samples diluted in PBS (once/day for 14 days). After treatment, the mice were sacrificed and the tumors removed and weighed. These experiments were repeated three times with three mice per each group.

For Lewis lung carcinoma therapy, mice were injected *im*. with 1×10^5 of Lewis lung carcinoma cells. Cyclophosphamide (30 mg/kg) was used *ip*. at day 8 after tumor application (positive control), individual substances were used from day 0 to day 14 after tumor application. The control group of mice (negative control) received *ip*. PBS daily. Each group held a minimum of five mice. At the conclusion of the experiment (day 14), mice were euthanized, their lungs were removed and fixed in 10% formalin, and the number of hematogenic metastases in lung tissue was estimated using a binocular lens at 8× magnification.

B16 melanoma cells were inoculated subcutaneously into shaved lateral flanks of the mice. The mice were treated by intraperitoneal injections of tested samples diluted in PBS once daily for 14 days. At the end of experiment, tumors were removed and weighed.

Results

Commercial samples can contain small amounts of biologically inert fillers. Even though these fillers are not expected to influence the biological activities of the samples, it is theoretically possible that the unknown amount of fillers might affect the dose of glucan used in the study, as the 100 mg/capsule might in reality mean that the capsule weights more than 100 mg (*i.e.*, 100 mg glucan plus fillers). Subsequent dilutions might in fact lower the used dose. To account for this possibility, we used Glucan #300, which is available as both a powder and a capsule, and compared the effects of several doses of powder and corresponding doses

Table II. *Effect of glucan dose on phagocytosis.*

Glucan	50 μg	100 μg	200 μg	400 μg
1	33.6±1.9	37.4±2.2*	38.9±2.4*	40.1±3.3*
2	31.8±1.7	35.8±3.0	37.2±2.1*	38.2±4.3*
3	33.8±2.7	38.9±3.1*	40.2±4.3*	42.2±2.7*
4	31.1±3.3	32.8±2.6	35.3±3.8	38.9±3.1*
5	35.6±1.9	38.9±2.2*	41.9±2.8*	43.9±3.2*
6	32.9±2.8	34.9±26	38.1±2.3*	40.1±3.5*
7	33.1±1.9	35.2±3.8	36.8±4.1	37.8±1.3*
8	38.9±1.8*	42.8±3.2*	44.9±2.1*	48.2±3.2*
9	37.6±2.7*	42.8±2.9*	47.8±3.1*	45.9±2.6*
10	36.1±2.2*	37.4±2.8*	40.4±3.1*	44.4±3.2*
11	33.1 ±3.8	34.9±3.1	36.1±1.8*	37.7±2.2*
12	37.1±4.2	39.1±2.8*	40.7±3.0*	40.1±3.5*
13	28.7±2.1	30.5±3.0	31.7±2.1	30.4±3.0
14	34.6±1.9	38.1±2.2*	38.2±2.4*	39.3±2.6*
15	31.6±2.9	33.8±2.8	35.0±3.2	38.2±1.9*
16	47.5±3.1*	52.5±3.5*	55.8±3.8*	55.0±4.0*

Control values (PBS) were 31.1±2.0. *Significant at $p<0.05$ level.

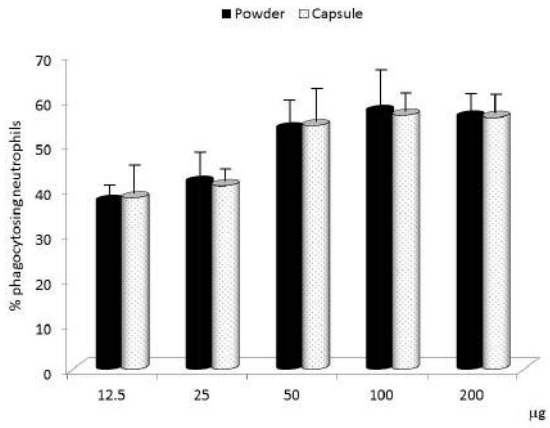

Figure 1. *Effects of addition of different doses on either glucan originated as powder (Powder) or used directly from the capsule (Capsule) on the phagocytosis of microparticles by peripheral blood neutrophils. Experiments were repeated three times, results are given as mean±SD.*

Table III. *Effect of glucan supplementation on IL-2 production.*

Glucan	IL-2 (pg/ml)
1	234.7±28.7
2	211.9±73.3
3	464.4±39.9
4	255.2±44.7
5	465.8±87.1
6	414.8±49.7
7	331.7±87.9
8	637.3±99.9
9	612.2±56.6
10	674.3±106.6
11	398.3±48.5
12	435.7±56.5
13	270.5±44.6
14	399.8±40.3
15	295.2±38.7
16.	801.1±102.7
PBS	0
Con A	1 011±301.2

All results are significant at $p< 0.05$ level.

taken from the capsule. Figure 1 summarizes the effects on phagocytic activity and shows that the results were identical even in the lowest dose used. However, as the amount of fillers can be different from company to company, we weighed the capsule contents and calculated the amount of glucan in all subsequent experiments.

Since a lipopolysaccharide (LPS) contamination might mask the effects of any biological response modifier, we checked the LPS contamination of our samples using a 10 μg/ml solution of polymyxin B. We prepared LPS-free glucans and tested them on phagocytosis. The results were identical to those using regular samples (data not shown); therefore, we concluded that LPS is not responsible for data observed in this study.

Table II shows the effects of tested glucans on phagocytosis of peripheral blood neutrophils. In order to better show the effects, we used four different doses (from 50 μg to 400 μg). Our results show clear dose dependency, but only in sufficiently active glucans. We found no activity at all in some glucans (sample #13), and others (*e.g.*, #4, #7 and #15) showed activity only in extremely high doses.

Glucans stimulate not only the cellular branch of immune reactions, but also the humoral part. We focused on formation of IL-2 by spleen cells. The production of this cytokine was measured after a 48 h *in vitro* incubation of cells isolated from PBS- or glucan-injected animals. Since the IL-2 secretion of control mice was always zero, all effects of glucan were statistically significant. The most active glucan was glucan #16, followed by glucans #10, #8, and #9 (Table III). None of the samples, however, reached the effects of positive control (Concanavalin A).

In the next part of our experiment, we focused on the use of glucan as an adjuvant using an experimental model of ovalbumin as an antigen. Individual samples were applied daily. Commonly used Freund's adjuvant was used as a positive control. The results are summarized in Table IV, demonstrating that most glucans stimulated the antibody formation, but glucans #1, #2, #7, #11, and #13 showed insignificant effects. The most active sample was #16 followed by samples #8, #10, and #6.

Table IV. *Effect of glucan supplementation on antibody formation.*

Glucan	% of control (ovalbumin only)
1	118.3±27.2
2	101.1±28.8*
3	252.2±20.1*
4	217.2±36.5*
5	241.1±31.7*
6	272.0±28.9
7	102.6±40.3*
8	293.0±38.5*
9	245.5±25.8*
10	272.3±29.2
11	142.3±34.4*
12	199.3±41.3
13	128.2±29.8*
14	177.4±30.5
15	155.7±43.8*
16	337.2±37.4
Ovalbumin + FA	486.4±44.8

Significant at p<0.05 level.

Table V. *Effect of glucan supplementation on suppression of lung cancer.*

Glucan	No. of metastases in lung
1	23.6±3.6
2	24.2±3.1
3	16.5±2.0*
4	22.6±2.9
5	18.1±1.5*
6	20.6±2.9
7	22.1±2.0
8	14.2±1.8*
9	17.2±3.5*
10	15.5±1.7*
11	21.6±2.2
12	16.6±1.7*
13	23.0±3.1
14	20.2±3.1
15	20.8±3.9
16	11.3±1.4*
PBS	25.3±2.0

*Significant at $p<0.05$ level.

In the last part of our comparative study, we focused on effects of glucans on inhibition of cancer growth. To be sure the effects of these samples reflected their potential anticancer properties, we used two different experimental models. With the first model, using a well-defined Lewis lung carcinoma model, we found that seven samples significantly lowered lung metastases (Table V). With the second, using a breast cancer model, we monitored the changes in tumor weight.

Table VI. *Effect of glucan supplementation on mammary carcinoma.*

Glucan	Tumor weight (mg)
1	564.8±77.8
2	601.3±69.9
3	373.5±58.9*
4	525.7±73.2
5	552.1±90.8
6	501.3±42.9*
7	559.4±83.9
8	401.9.±53.4*
9	512.7±98.3
10	389.0±51.7*
11	553.7±73.1
12	447.1±44.3*
13	590.2±66.0
14	501.7±40.8*
15	520.2±77.1
16	302.4±57.2*
PBS	681.4±66.8

*Significant at $p<0.05$ level.

Table VII. *Effect of glucan supplementation on weight of some organs and primary tumors in melanoma-treated mice.*

Glucan	Liver	Lung	Spleen	Tumor
1	1.66±0.20	0.21±0.09	0.32±0.07	0.51±0.11
2	1.64±0.23	0.20±0.07	0.31±0.08	0.49±0.12
3	1.34±0.04*	0.19±0.05	0.36±0.11	0.44±0.14
4	1.60±0.25	0.22±0.06	0.35±0.12	0.48±0.24
5	1.64±0.27	0.17±0.10	0.34±0.19	0.42±0.19
6	1.31±0.08*	0.18±0.09	0.28±0.05*	0.37±0.07*
7	1.62±0.22	0.22±0.09	0.35±0.08	0.47±0.17
8	1.30±0.11*	0.23±0.05	0.32±0.12	0.30±0.11*
9	1.78±0.14	0.25±0.09	0.33±0.11	0.29±0.08*
10	1.67±0.17	0.27±0.09	0.30±0.16	0.55±0.11
11	1.59±0.14	0.22±0.07	0.32±0.08	0.52±0.12
12	1.19±0.10*	0.21±0.08	0.22±0.10	0.46±0.11
14	1.39±0.14*	0.24±0.06	0.35±0.12	0.35±0.15*
15	1.77±0.23	0.25±0.04	0.36±0.11	0.52±0.14
16	1.22±005*	0.18±0.02	0.21±0.5*	0.33±0.08*
PBS	1.85±0.10	0.22±0.03	0.37±0.06	0.60±0.13
Control	1.12±0.06	0.16±0.01	0.11±0.01	-

Data represent the mean±SD values. Control: Untreated control mice; PBS: melanoma-challenged mice treated with PBS. *Significant against PBS-treated group at $p<0.05$ level. Weight (g).

Our results showed that the individual glucans differed in their activity towards breast or lung cancer suppression and only samples #3, #8, #10, #12, and #16 were the same samples as in case of lung cancer growth. In all other cases, the effects were either not statistically significant or there were no effects at all (Table VI).

Administration of melanoma cells resulted in a significant weight increase of individual organs such as liver, lung, or spleen (Table VII). Six glucans managed to lower the weight of liver and two of those were also active in the lung. However, none of tested samples had any effect on spleen weight. Five samples successfully lowered the weight of tumors; the most active samples were #8 and #16.

Discussion

Glucans are natural polysaccharides consisting of linked glucose molecules. In nature, glucans form a major structural component of the cell wall of numerous organisms such as fungi, bacteria, and yeasts. In addition, glucan can be found in plants; *e.g.*, oat and barley contain glucan as a part of endosperm.

Polysaccharides such as glucans have been studied for almost a century. Almost 80 years ago, Shear and coworkers isolated from the culture of *Serratia marcescens* a substance causing necrosis of tumors (19). Due to the justified distrust of bacterial sources of polysaccharides, attention gradually shifts to much less dangerous microorganisms, first of all to yeasts. Their intact cells could be, of course, also pyrogenic (20). This pyrogenicity, given mostly by contaminating proteins, is negligible compared to the other effects. Pure isolated polysaccharides are apyrogenic and their biological effects have nothing to do with it, supporting the need for use highly purified glucans. Authors seeking more information on pyrotherapy and polysaccharides should see a recent review by Novak and Vetvicka (21).

Due to the uncertainty of using lipopolysaccharide, our attention focused on zymosan, but even that was not completely without problems. Even though zymosan was able to significantly stimulate nonspecific immune response, it was not clear which of its components was responsible for that activity. Zymosan is not a pure polysaccharide but rather a crude insoluble product, containing about 50% of β-glucan and also around 17% of mannan, 14% of a protein, and some impurities (22). Numerous subsequent studies demonstrated that the active component, responsible for the primary effect of zymosan, is branched polysaccharide β(1-3),β(1-6)-D-glucan (23). Since that time, glucans have become the most studied natural immunomodulators and, due to the many ongoing human clinical trials, have a very strong chance of becoming an officially approved drug in Western medicine.

Despite tens of thousands published papers on biological activities of glucan, it is often difficult to compare the effects as the majority of authors used glucans differing in source, size, or physicochemical properties. Despite numerous interesting reviews summarizing the current knowledge of glucan activities (24-27), the best compare individual glucans against each other using identical experimental designs. In our previous studies, we directly compared over 50 individual glucans (12-16, 18). However, the number of commercially available glucans has multiplied in many countries since, prompting us to compare the new batch of available glucans with glucan #300, which consistently showed the highest activities in the previous studies. In the present study, we used some of the same activities (*i.e.*, phagocytosis, IL-2 production, and antibody response) and added effects on three different types of cancer, as glucan is already used in cancer treatment (11) and most ongoing clinical trials are currently focused on the anti-cancer activities of glucan.

Phagocytosis is often the first test of glucan activities, as glucans were originally considered to be nonspecific stimulators of innate immunity. Phagocytosis is usually increased by glucan supplementation, and the true glucan effects are manifested *via* receptor-specific interaction (28). Like in previous studies, we employed synthetic 2-hydroxyethyl methacrylate microparticles known for their minimal nonspecific adhesion to the cell membrane (17), thus limiting false positivity. Our results showed that one-half of the tested samples had no activity at the common dose of 100 μg and one sample had no activity even at the highest dose of 400 μg. On the other hand, the best glucans showed statistically significant activity even at the lowest dose.

Immunostimulators in general and glucans in particular stimulate secretion of various cytokines such as IFN-γ, IL-1, IL-2, and TNF-α (29, 30, 31). In our study, we measured the production of IL-2 by spleen cells. Without any stimulation, the release of IL-2 by these cells is zero, therefore all samples showed significant stimulation (Glucan #300 showing the highest effects). Concanavalin A, serving as a positive control, had even stronger effects.

After overcoming the theory that glucans are only nonspecific stimulators of defense reaction, the common dogma stated that they act on cellular immunity only. Lately, attention is beginning to focus on possible effects on humoral immunity (32), and glucans are now considered to have important potential as a part of vaccines. Our results further confirm that high quality glucans will stimulate antibody response, which, together with the findings of potentiation of the effect of anti-cancer monoclonal antibodies (33), led us to focus on cancer.

Glucan effects on cancer inhibition are well established (7, 34, 35), but only rarely somebody tests the same glucans on several different cancer models. We used three different models, breast and lung cancers and melanoma. Our data showed that glucan supplementation significantly reduced both the weight of primary tumors and the number of lung colonies. In our previous study of testing glucan effects on melanoma, we clearly demonstrated that glucan inhibited the damage to blood cells and potentiated the effects of regular chemotherapy. In addition, these effects were manifested *via* activation of NK cells (36). Many glucans showed the ability

to suppress cancer growth, but this activity varied based on cancer model and only glucans #16 and #8 had significant anti-cancer activity in all three models.

In summary, this study is a follow-up of our three previous studies, testing 43 different glucans. From these experiments, we can conclude that on one hand, glucans can have substantial effects on all branches of the immune system, but on the other hand, not all glucans are created equal. Similar to our older comparative studies, we used glucans isolated from yeast, mushroom, algae, and oat. However, no clear correlation between function and other characteristics, such as source or solubility, could be reached. The differences between activities of our commercially available glucans might be an explanation for the sometimes confusing results found in the literature. In all tests employed in our study, Glucan #300 was the most active.

Conflicts of Interest

No conflicts of interests exist for the Authors.

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