

Research Article

Comparison Of Immunological Effects Of Commercially Available β -Glucans.

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Abstract

Beta glucan belongs to the most promising natural immunomodulators. However, despite decades of intensive research, its biological activity is still not fully understood. One of the causes might be the difference between individual glucans. In this study we compare nine different commercially available glucans for their activity in phagocytosis, IL-2 production, antibody formation and cancer growth reduction. Our data clearly showed the significant differences among these glucans and further confirmed that even if most glucans have strong stimulating effects on most aspects of the immune system, it is necessary to choose the right one.

Keywords: Glucan; phagocytosis; IL-2; antibody; cancer

INTRODUCTION

Beta glucan (glucan) is a polysaccharide — a chain of glucose molecules linked in specific ways (β -1,3 and β -1,4 or β -1,6 bonds, depending on the source).¹ Their original biological function is support of rigidity and protection to the cell wall. After decades of intensive research, scientists found numerous biological effects including physiological, metabolic and immunostimulating actions.

Oat and barley glucans increase intestinal viscosity, bind bile acids, and reduce cholesterol reabsorption — leading to lower LDL-C and improved glycemic response.² Some of these effects were later found also in yeast-derived glucans.³ Lately, most attention is focused on immunomodulatory properties of various glucans.^{4,5} β -glucan has been found to affect several types of immune cells, including macrophages, natural killer cells, neutrophils, dendritic cells and lymphocytes. For recent summary of immunomodulatory effect and biological significance of glucans see.⁶

Despite over 50,000 scientific studies published in various journals, the individual results are still not fully compatible. The main reason might be the fact that individual glucans differ in numerous characteristics, such as molecular weight,

branching, and solubility, making it difficult to unify the described biological effects.⁶

So far, only a few papers compared individual immunostimulators.^{7,8,9,10} We would like to point out that the discrepancies between the effects of different glucan can be solved only by direct comparison. Based on the limited published comparisons, we decided to compare numerous commercially available glucans.

RESULTS

Animals

Female, 8-week-old BALB/c mice were purchased from the Jackson Laboratory (Bar Harbor, ME). All animal work was done according to the University of Louisville IACUC protocol. Animals were sacrificed by CO₂ asphyxiation followed by cervical dislocation.

Material

All glucans were either donated or purchased from the manufacturers or distributors as shown in **Table 1**. Ovalbumin, polymyxin B, cyclophosphamide, and Wright stain were purchased from Sigma (St Louis, MO, USA).

Table 1. List of samples used in the study

#	Name	Source	Manufacturer
1	Immune Restore+	Yeast	NeurobiologiX
2	ImmunotiX 250	Yeast	Xymogen
3	Beta Xym	Agrobacterium	U.S. Enzymes
4	Innate Immune Support	Mushroom	Pure Encapsulations
5	Beta Glucans	Yeast	Dr. Mercola Formulas
6	Beta Glucan	Yeast	Jarrow Formula
7	Beta Glucan	Mushroom	Now
8	Mushroom Immune	Mushroom	Life Extension
9	#300 Glucan	Yeast	Transfer Point

Cell line

Lewis lung carcinoma cells (obtained from Dr. G. Ross, University of Louisville, Louisville, KY, USA) were maintained in at 37 °C in a humidified atmosphere supplemented with 5% CO₂ in RPMI 1640 medium (Sigma) supplemented with 10% FCS (Sigma).

Phagocytosis

Phagocytosis of 2-hydroxyethyl methacrylate particles was described previously.¹¹ Briefly, 0.1 ml of peripheral blood from mice treated with various doses of glucan or PBS was incubated in vitro with 0.05 ml of microspheres (5x10⁸/ml) 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. All experiments were performed in triplicate. At least 300 cells were examined in each experiment.

IL-2 secretion

Purified spleen cells (2x10⁶/ml in RPMI 1640 medium supplemented with 5% FCS) obtained from mice treated with 100 µg glucan or PBS were added into wells of a 24-well tissue culture plate. Cells were incubated for 48 hrs in a humidified incubator (37 °C, 5% CO₂/95% air). Addition of 1 µg of Concanavalin A (Sigma) was used as a positive control. At the endpoint of incubation, supernatants were collected and tested for the presence of IL-2 using a Quantikine mouse IL-2 kit (Abcam, Cambridge, Great Britain).

Antibody formation

The technique using ovalbumin as an antigen was described earlier.⁷ Mice were injected twice (14 days apart) with 100 µg of albumin and the serum was collected on day 21. 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.

Lung carcinoma therapy

First, mice were injected *im.* with 1x10⁵ of Lewis lung

carcinoma cells. Cyclophosphamide (30 mg/kg) was used *ip.* at day 8 after tumor application (positive control), individual substances were used daily from day 0 to day 14 after tumor application. The control group of mice received *ip.* PBS daily. Each group held a minimum of five mice. At the conclusion of the experiment (day 14), mice were euthanized, lungs were removed, fixed in 10% formalin, and the number of hematogenic metastases in lung tissue was estimated using a binocular lens at 10x magnification as described earlier.¹²

Results

Commonly, commercial samples of any supplement contain small amounts of biologically inert fillers with no expected to effects on the biological activities of the supplements. However, one needs to keep on mind that it is theoretically possible that the unknown amounts of fillers might affect the dosing of active ingredience, as the 10 mg/capsule might in reality mean that the capsule weights more, i.e., 10 mg of active material plus fillers. To account for this possibility, we previously tested one glucan available as both a powder and a capsule, and compared the effects of several doses of powder and corresponding doses taken from the capsule and found that the results were identical even in the lowest dose used.¹² However, as the amounts of fillers differ from company to company, we weighed the capsule contents and calculated the amount of glucan in all experiments.

Phagocytosis is the process by which phagocytes (monocytes, neutrophils, macrophages) engulf and digest foreign particles, microbes, or debris. Phagocytosis is a key process of the innate immune system — it represents the first line of defense. Beta-glucans stimulate phagocytosis by binding to various membrane receptors including CR3 and dectin-1 receptors on macrophages and neutrophils which subsequently enhances immune readiness and helps the body respond faster to infections.

Because phagocytosis is such common immunological reaction, stimulation of phagocytosis is usually the first on line in testing of natural immunomodulator. It was established that if the immunomodulator does not influence phagocytosis, its effects on other fascets of immune reactions

will be neglectable. Using a model of synthetic polymeric 2-hydroxyethylmethacrylate microspheres, we measured the phagocytic activity after two weeks feeding with tested substances. To better understand the effects of glucan, we evaluated six different doses, from 25 to 800 mg/day. Our results are shown in **Table 2** showed that individual glucans significantly increased phagocytic activity in almost all concentrations, only in concentration 25 mg/ml were most of glucans without significant activity. Only few glucans showed fully dose-dependent activity. Most active beta glucan was glucan #9.

Table 2. Effects of tested samples on phagocytic activity

Sample	25	50	100	200	400	800
1	36.4 ± 1.5	34.9 ± 3.8	43.0 ± 3.3	44.7 ± 3.1	50.1 ± 4.6	49.1 ± 2.5
2	35.1 ± 1.7	37.9 ± 2.2	39.1 ± 2.0	42.8 ± 2.4	46.2 ± 2.8	47.8 ± 4.2
3	30.9 ± 2.0	33.4 ± 3.2	36.9 ± 2.5	40.1 ± 4.4	43.3 ± 2.0	42.8 ± 3.3
4	33.3 ± 1.8	37.2 ± 2.0	39.9 ± 2.7	42.8 ± 3.1	46.3 ± 4.1	48.0 ± 2.9
5	36.2 ± 1.9	40.3 ± 2.7	41.7 ± 3.5	44.9 ± 2.7	47.8 ± 3.9	47.1 ± 3.0
6	33.4 ± 1.5	38.2 ± 2.5	40.0 ± 1.9	43.2 ± 3.4	45.9 ± 2.8	48.3 ± 3.1
7	36.7 ± 1.7	37.3 ± 2.2	45.2 ± 3.3	41.6 ± 4.7	45.8 ± 3.1	43.8 ± 5.2
8	31.9 ± 2.0	35.8 ± 2.1	40.1 ± 1.8	42.2 ± 4.1	44.9 ± 5.2.	44.1 ± 2.0
9	40.1 ± 2.6	44.4 ± 2.6	53.9 ± 2.3	58.8 ± 3.1	56.6 ± 2.7	60.2 ± 4.4
PBS		28.7 ± 1.1				

3 mice/group, glucan *i.p.*

% phagocytosing neutrophils

In the next step we focused on humoral immunity and measured production of IL-2. IL-2 levels were measured after a 72 hr *in vitro* incubation of spleen cells isolated from control and glucan-treated animals. Since the secretion of IL-2 by non-stimulated cells was always a zero, every tested material showed significant effects (**Table 3**). The most active glucan was again glucan #9, which at 100 mg/ml dose showed higher activity than the rest of glucans as 800 mg/ml.

Table 3. Effect of various samples on IL-2 production

Sample	25	50	100	200	400	800
1	48.5 ± 3.5	104.8 ± 13.2	138.0 ± 9.3	194.5 ± 13.4	190.8 ± 24.0	243.1 ± 12.9
2	131.1 ± 9.7	199.3 ± 20.2	251.1 ± 21.2	324.7 ± 20.4	445.3 ± 21.7	442.1 ± 29.3
3	66.8 ± 7.1	99.5 ± 13.0	136.1 ± 8.5	243.4 ± 13.2	248.3 ± 12.1	344.7 ± 23.8
4	137.7 ± 6.8	273.4 ± 12.0	299.7 ± 13.7	344.5 ± 15.2	440.5 ± 17.1	508.1 ± 22.6
5	99.3 ± 8.2	146.6 ± 11.1	249.3 ± 9.8	277.8 ± 10.7	379.6 ± 15.2	440.3 ± 23.1
6	213.5 ± 9.7	283.3 ± 12.5	341.2 ± 11.6	340.0 ± 13.6	405.7 ± 16.8	443.4 ± 21.4
7	106.1 ± 7.7	172.4 ± 11.4	241.5 ± 9.8	249.7 ± 14.8	316.1 ± 23.0	403.1 ± 24.3
8	101.2 ± 6.1	158.5 ± 6.6	242.4 ± 8.8	340.1 ± 9.9	346.9 ± 15.3	404.4 ± 29.2
9	340.1 ± 12.5	437.0 ± 19.7	801.5 ± 29.9	845.9 ± 20.6	911.2 ± 30.6	910.8 ± 28.1
PBS		11.7 ± 0.3				

Results represent mean ± SD. All results are significant at $P < 0.05$ level.

As an additional experimental model, we measured the possible effects on antibody response. We used the test employing immunization with ovalbumin as an antigen. Mice were fed with 100 mg/ml of individual glucans and injected twice (two weeks apart) with 100 mg of albumin and the serum was collected 7 days after last injection. Most samples stimulated antibody formation, with the glucans #2 and #9 showed the strongest activity. Sample #1 showed no activity at all (**Table 4**).

Table 4. Effect of tested samples on antibody formation

Sample	% of control (albumin only)
1	117.4 ± 11.2
2	428.5 ± 23.2*
3	125.9 ± 22.0
4	133.9 ± 15.6*
5	236.5 ± 19.6*

6	236.7 ± 12.9*
7	296.7 ± 19.8*
8	151.5 ± 12.8*
9	456.3 ± 22.1*
Ovalbumin + FA	520.1 ± 31.4

In the last step, we evaluated possible role of tested glucans in cancer inhibition. Using a well-established model of Lewis lung carcinoma cells, we previously showed that cyclophosphamide administered in the used dose caused 70% inhibition of the number of lung metastases in comparison to the control group.¹³ Our data summarized in **Table 5** show that some glucans significantly lowered the number of lung metastases, with samples #2, #7, #8 and #9 showing the significant effects. Glucan #9-caused inhibition was similar to the effects of cyclophosphamide.

Table 5. Effect of tested samples on suppression of lung cancer growth

Sample	Number of lung metastasis
1	22.1 ± 1.9
2	18.2 ± 3.9*
3	25.0 ± 4.4
4	23.5 ± 5.0
5	26.1 ± 1.9
6	20.1 ± 2.5
7	16.6 ± 3.8*
8	19.5 ± 2.4*
9	11.3 ± 1.5*
PBS	27.3 ± 3.0

Results represent mean ± SD. *Results are significant at $P < 0.05$ level.

DISCUSSION

Cheap and most of all effective natural immunomodulators represent a holy grail of alternative medicine for decades. Some of these natural materials are being extensively studied for long time. Glucans are known for an impressive number of peer-reviewed scientific papers reaching 50,000. They support and enhance the immune system by activating both innate and adaptive immune cells like macrophages, NK cells, and T and B cells. They function as immunomodulators that strengthen the body's defenses against pathogens, support immune response to infections, improve vaccine effectiveness, and may offer anti-tumor benefits.¹⁴ On the other hand, some potential immunomodulators were tested only perfunctorily or the results are confusing. As an example of the latter situation can serve Echinacea, as its activity significantly differ based on part of plant used for isolation.¹⁵ With no consensus which part to use, it is not surprising that the results are not consistent and clinical trials failed. Despite high amount of published papers on biological activities of glucan, it is often difficult to compare the effects as individual authors used glucans differing in source, size, and/or physicochemical properties. Despite numerous interesting reviews summarizing the current knowledge of glucan actions,^{16,17} we believe that the best option is to compare individual glucans using identical experimental

designs. In our previous studies, we directly compared over 60 individual glucans.^{18,19,8} However, with the number of commercial glucans still multiplying all over the world, we decided to compare the new batch of available glucans with Glucan #300, which previously showed the highest activities. We decided to use three methods covering all main aspects of immune reaction – native immunity (phagocytosis) and acquired immunity (cytokine production and antibody response). In addition, we added effects on cancer growth. Glucan's effects on cancer growth are already well established,^{20,21,22,23} therefore we tested our samples on mouse lung cancer model. The last part of our ongoing investigation of commercial glucans clearly demonstrated that several differences in biological activities exist.

Two conclusions can be reached from our study. First, all glucans showed biological activity, although not in every reaction. This is particularly true where there is no basal level of activities (e.g., IL-2 production or antibody formation), all or at least most glucans showed significant activity. And second – one sample, Glucan #300, was consistently the most active glucan from all. Future research should try to solve mechanisms, establish standardization, and explain existing gaps to unlock therapeutic possibilities of glucan.

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