

β 1,3-Glucan: Silver Bullet or Hot Air?

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Abstract: β -Glucans belong to a group of biologically active natural compounds called biological response modifiers. These substances represent highly conserved structural components of cell walls in yeast, fungi, grain and seaweed. The effects of β -glucan on immune reactions are well established however direct comparisons of the biological activities of several individual glucans are extremely rare. As this paper will show, we tested sixteen different glucans and evaluated the possibility whether individual glucans will be similarly active against each of the tested biological properties or if each glucan will affect different reactions. No direct connection between source and immunological activities was found. Based on our results, we can conclude that highly purified and highly active glucans have pleotropic effects, whereas poorly isolated glucans have only average (if any) biological effect.

Keywords: Glucan; phagocytosis, IL-2, immunity, antibodies.

INTRODUCTION

β 1,3-Glucan's role as an immunomodulator has been well documented for over 50 years. Initial interest in the immunomodulatory properties of polysaccharides was raised after experiments revealed that a crude yeast cell preparation stimulated macrophages *via* activation of complement [1]. Further work identified the immunomodulatory active component as β 1,3-glucan [2]. β -Glucans show notable physiological effects; this is their most important quality and the reason why so much attention has been focused on them. Numerous studies (currently more than 6,000 publications) have subsequently shown that β 1,3-glucans, either particulate or soluble, exhibit immunostimulating properties, including antibacterial and anti-tumor activities (for review see [3,4]).

There are various natural sources of β -glucans; however, they are most often prepared from fungal cell walls. Baker's yeast is the most common and likely the best raw material for glucan extraction. Additional sources of glucan involve mushrooms, grain and seaweed. Diverse data on comparison of structure, molecular size, and biological effects can be found in literature. For example, the antitumor activity of schizophyllan is supposedly conditioned by the triple helix presence and a molecular weight higher than 100 kDa. It is more than likely that the triple helix structure is not the sole effective form of β -glucan, because alkalic treatment, used in most isolation procedures, destroys this structure. In addition, the most recent opinions do not confirm the established ideas of the necessity of high molecular mass and branching of biologically active β -glucans.

Glucan is not only the most studied natural immunomodulator, it is also extremely successful commercially with numerous manufacturers/resellers active in every country. Because of the lack of comprehensive reviews comparing the

biological effects of glucans isolated from various sources, and despite extensive investigations, no final conclusion has been reached. Therefore, there is not a single research paper willing to state that one source of glucan is better than another. In addition, numerous concentrations and routes of administration have been tested, including intraperitoneal, subcutaneous, and intravenous applications. This takes us back to the original question: which, out of dozens if not hundreds of individual glucans on the current market, is the best one? Which one has superior biological and/or immunological properties? For years, there was a controversy between the notions that water-insoluble glucans show only little biological activity, whereas soluble glucans are highly active. Similar questions whether orally-given glucan is as active as injected one has been solved only recently [5]. Based on limited published comparisons of individual glucans [5-8], we decided to compare numerous commercially available glucans.

MATERIALS AND METHODOLOGY

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.

Material

Individual glucans were purchased from the manufacturers or distributors as shown in Table 1.

Cell Lines

Human neutrophil cell line HL-60 and human myeloid cell line U937 were obtained from the ATCC (Manassas, VA). The cell lines were maintained in RPMI 1640 (Sigma Chemical Co., St. Louis, MO) medium containing HEPES (Sigma) buffer supplemented with 10% heat-inactivated FCS (Hyclone Lab., Logan, UT), 100U/ml penicillin (Sigma) and 100 μ g/ml streptomycin (Sigma), in plastic disposable tissue culture flasks at 37 °C in a 5% CO₂/95% air incubator.

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Table 1. Types of Glucan Used in this Study

| Glucan | Source | Solubility | Manufacturer | Reference |
|-------------------|----------|------------|--|-----------|
| Maitake Gold | Mushroom | Soluble | NutraGenesis, Brattleboro, VT | [6] |
| Krestin | Mushroom | Soluble | Kureha Corp, Japan | [5] |
| Aktival | Yeast | Insoluble | Farmal, Croatia | |
| Immutol | Yeast | Insoluble | Biotec ASA, Norway | [7] |
| Now Glucan | Yeast | Insoluble | Now Foods, Bloomingdale, IL | [7] |
| | Mushroom | Soluble | | |
| Sweet Beta Glucan | Yeast | Soluble | K2 Global, Las Vegas, NV | |
| Beta Right 101 | Yeast | Insoluble | Biothera, Eagan, MN | [26] |
| Epicor | Yeast | Insoluble | Vitamin Research Products, Carson City, NV | |
| NSC | Yeast | Insoluble | Nutritional Scientific Corp., Liberty, TX | [27] |
| Glucagel | Barley | Soluble | PolyCell Technologies, Crookston, MN | [7] |
| Better Immunity | Yeast | Insoluble | Young Again Nutrients, Magnolia, TX | |
| Macroforce | Yeast | Insoluble | ImmuneDyne, Houston, TX | [28] |
| Solgar | Yeast | Insoluble | Solgar, Leonia, NJ | |
| Wellmune | Yeast | Insoluble | Biothera, Eagan, MN | [26] |
| Swanson | Yeast | Insoluble | Swanson Health Products, Fargo, ND | |
| Glucan #300 | Yeast | Insoluble | Transfer Point, Columbia, SC | [7] |

Phagocytosis

The technique employing phagocytosis of synthetic polymeric microspheres was described earlier [9,10]. Briefly: peripheral blood cells or isolated peritoneal cells were incubated *in vitro* with 0.05 ml of 2-hydroxyethyl methacrylate particles (HEMA; 5×10^8 /ml). The test 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, glucan/resveratrol or PBS (control). All experiments were performed in triplicate. At least 200 cells in 60 high power fields were examined in each experiment.

IL-2 Production

Purified spleen cells (2×10^6 /ml in RPMI 1640 medium with 5% FCS) from mice injected with glucan were added into wells of a 24-well tissue culture plate. After addition of 1 µg of Concanavalin A, cells were incubated for 48 hrs in a humidified incubator (37° C, 5% CO₂). 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).

Antibody Formation

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 were getting daily

ip. injections of glucan. Level of specific antibodies against ovalbumin was detected by ELISA. As positive control, combination of ovalbumin and Freund's adjuvant was used.

Superoxide and Nitrite Production

Cells were incubated in a final volume of 200 µl of medium containing 0.1 % gelatin and 100 µM cytochrome C (Sigma, St. Louis, MO, USA). Mice were challenged with 100 µg of individual glucans 24 hrs earlier. Cell lines were incubated with 1 µg/ml of glucans for 24 hrs. For the superoxide production, the reaction was initialized by addition of 5 ng/ml PMA (Sigma). After gentle mixing, the absorbance was measured 30 minutes after incubation at 37° C using multiwell spectrophotometer at 550 nm. Results are expressed as nanomoles of cytochrome C reduced/ 2.5×10^5 cells/30 minutes, after subtraction of the SOD and spontaneous release controls [11].

For a nitrite (NO₂⁻) formation we used a technique described by Green and Nacy [12] with LPS (Sigma) as triggering agent.

RESULTS

The number of individual glucans is almost as great as the number of sources used for their isolation. The rationale for this combination of glucan samples was not only their commercial availability and success, but most importantly, our effort to include both soluble and insoluble glucans and also glucans isolated from different sources. Sixteen different glucans used for our comparison represented glucans available both on the U.S. and international markets. We

originally started with almost 50 different glucans, however for further testing we narrowed our choice to the sixteen ones. Information about individual types and manufacturer/distributors are given in Table 1.

β-Glucans are generally considered to be potent stimulators of cellular immunity, with macrophages and neutrophils being the most important target. Not surprisingly, we started our evaluation of glucan activities by phagocytosis. We used the synthetic polymeric microspheres HEMA, since their use, dose and timing is already well established in glucan studies [13,14]. Results summarized in Table 2 show the effects of glucan samples on phagocytosis of synthetic particles by peripheral blood neutrophils. Our data suggested dose dependency, but only in sufficiently active glucans. Some glucans were either active only in extremely high doses (Sweet Beta Glucan) or showed only small and non-significant stimulation of phagocytosis (Epicor, Better Immunity, Macroforce, Solgar, Swanson). In general, only Glucan #300 stimulated the reaction even at the lowest dose.

Internalization of material is just one step in the complex process of phagocytosis. Another step is connected with a burst of metabolic activity and involves production of active oxygen species. In order to further evaluate the effects of tested glucans on phagocytosis, we decided to measure the production of two important molecules—superoxide anion and nitrite oxide. To be sure that we cover all possible effects, we used both fresh mouse neutrophils and two established cell lines— neutrophil cell line HL-60 and monocyte cell line U937. Data summarized in Table 3 showed that whereas almost all glucans stimulated superoxide anion pro-

duction (with exception of Aktival), there were strong differences among individual samples with the most active glucans being Glucan #300, Wellmune, Maitake Gold, Krestin and Glucagel. Evaluation of nitrite oxide showed similar results (Table 4). Surprisingly, in this case Aktival showed strong effects.

Evidence of the immunomodulating activity was also demonstrated through effects on the production of IL-2 by spleen cells (Table 5). The production of IL-2 was measured after a 48 hr *in vitro* incubation of spleen cells isolated from control and glucan-treated mice. Since the secretion of IL-2 by non-stimulated splenocytes (PBS) was always zero, every tested glucan showed significant stimulation of IL-2 production, with the most active glucans being Glucan #300, Wellmune, Maitake Gold, Beta Right and Krestin. None of the glucans, however, reached the effects of Concanavalin A; only Glucan #300 showed statistically the same effects.

Next, we focused on the use of glucan as an adjuvant. As an experimental model, we used immunization with ovalbumin. Glucans were applied together with two intraperitoneal doses of antigen, a commonly used Freund's adjuvant was used as additional positive control. The results (Table 6) showed that despite the fact that all glucans potentiated the antibody response, significant differences between individual samples existed. The highest activity was measured in case of glucagel, Wellmune, Glucan #300 and Beta Right.

DISCUSSION

Despite the extensive amount of scientific reports about glucans and their biological activities, most of the studies are

Table 2. Effect of Dose on Stimulation of Phagocytosis

| Dose (μg) | 25 | 50 | 100 | 200 | 400 | 800 |
|-------------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Maitake Gold | 34.7 ± 2.4 | 35.5 ± 3.1 | 38.9 ± 3.1* | 45.4 ± 4.1* | 46.0 ± 4.0* | 46.2 ± 4.2* |
| Krestin | 36.2 ± 1.1 | 37.8 ± 2.1* | 44.9 ± 2.5* | 44.1 ± 3.5* | 48.5 ± 4.4* | 49.2 ± 3.9* |
| Aktival | 31.1 ± 1.6 | 34.6 ± 2.0 | 38.9 ± 3.0* | 40.2 ± 3.3* | 41.1 ± 4.7* | 40.5 ± 3.7* |
| Immutol | 32.3 ± 2.1 | 35.5 ± 2.9 | 39.6 ± 3.3* | 41.3 ± 4.1* | 41.7 ± 3.5* | 43.0 ± 4.1* |
| Now Glucan | 36.2 ± 2.1 | 39.8 ± 1.1* | 46.2 ± 2.5* | 41.4 ± 3.0* | 44.1 ± 3.6* | 42.9 ± 3.8* |
| Sweet Beta Glucan | 30.1 ± 2.2 | 31.2 ± 1.8 | 33.7 ± 2.3 | 34.1 ± 2.0 | 33.8 ± 2.4 | 37.8 ± 3.2* |
| Beta Right 101 | 35.1 ± 0.8 | 42.7 ± 2.7* | 44.9 ± 3.0* | 47.1 ± 3.3* | 48.2 ± 3.8* | 48.1 ± 2.1* |
| Epicor | 30.1 ± 1.1 | 28.5 ± 3.2 | 30.6 ± 2.4 | 33.2 ± 3.3 | 35.6 ± 3.8 | 34.9 ± 4.6 |
| NSC | 29.9 ± 3.3 | 32.9 ± 2.5 | 31.6 ± 3.2 | 33.7 ± 3.8 | 35.7 ± 3.5 | 36.6 ± 2.9* |
| Glucagel | 30.1 ± 1.7 | 32.5 ± 2.8 | 35.6 ± 2.1 | 37.1 ± 2.6* | 36.9 ± 3.0* | 38.0 ± 2.9* |
| Better Immunity | 32.1 ± 0.8 | 33.6 ± 1.7 | 33.8 ± 3.0 | 35.5 ± 2.9 | 36.1 ± 4.2 | 34.2 ± 3.2 |
| Macroforce | 29.7 ± 3.0 | 30.6 ± 0.9 | 32.1 ± 2.8 | 33.6 ± 2.5 | 32.9 ± 5.1 | 33.8 ± 2.7 |
| Solgar | 30.1 ± 3.1 | 31.2 ± 2.2 | 32.5 ± 4.4 | 31.1 ± 2.4 | 34.8 ± 2.5 | 33.2 ± 2.9 |
| Wellmune | 28.5 ± 2.8 | 35.0 ± 2.1 | 42.1 ± 3.0* | 44.2 ± 3.1* | 44.7 ± 2.5* | 45.9 ± 3.7* |
| Swanson | 27.9 ± 1.2 | 30.1 ± 2.3 | 33.5 ± 2.7 | 35.2 ± 2.2 | 34.7 ± 1.9 | 35.9 ± 4.1 |
| Glucan #300 | 44.1 ± 2.5* | 48.8 ± 2.1* | 55.7 ± 3.2* | 56.1 ± 2.9* | 55.9 ± 3.2* | 60.9 ± 4.0* |

Control values (PBS) were 30.4 ± 2.7. *Significant at <0.05 level.

Table 3. Effects of Glucan on Superoxide Anion Production

| Glucan | superoxide anion | (nanomoles per 2.5 x 10 ⁵ cells) | |
|-------------------|-------------------|---|---------------|
| | Mouse neutrophils | HL-60 | U937 |
| Maitake Gold | 1.38 ± 0.22** | 1.45 ± 0.33** | 1.54 ± 0.25** |
| Krestin | 1.17 ± 0.33** | 1.02 ± 0.26** | 1.11 ± 0.15* |
| Aktival | 0.36 ± 0.09 | 0.27 ± 0.11 | 0.65 ± 0.23* |
| Immutol | 0.67 ± 0.12* | 0.67 ± 0.24* | 1.02 ± 0.34* |
| Now Glucan | 1.27 ± 0.09** | 0.98 ± 0.11** | 1.07 ± 0.22* |
| Sweet Beta Glucan | 0.44 ± 0.07* | 0.30 ± 0.06* | 0.66 ± 0.15* |
| Beta Right | 1.12 ± 0.15** | 1.23 ± 0.16** | 1.30 ± 0.25** |
| Epicor | 0.49 ± 0.16* | 0.34 ± 0.24* | 0.45 ± 0.11 |
| NSC | 0.44 ± 0.15 | 0.35 ± 0.08* | 0.71 ± 0.16* |
| Glucagel | 1.49 ± 0.33** | 1.50 ± 0.41** | 1.43 ± 0.38** |
| Better Immunity | 0.78 ± 0.12* | 0.98 ± 0.16* | 1.11 ± 0.18* |
| Macroforce | 0.56 ± 0.20* | 0.55 ± 0.09* | 0.97 ± 0.24** |
| Solgar | 0.37 ± 0.06 | 0.22 ± 0.04* | 0.69 ± 0.17* |
| Wellmune | 1.01 ± 0.23* | 0.99 ± 0.18** | 1.24 ± 0.20** |
| Swanson | 0.87 ± 0.17* | 0.25 ± 0.03* | 0.77 ± 0.12* |
| Glucan #300 | 1.69 ± 0.34** | 1.55 ± 0.27** | 1.81 ± 0.35** |
| PBS | 0.23 ± 0.07 | 0.11 ± 0.02 | 0.33 ± 0.06 |

*Significant stimulation of superoxide anion production at P<0.05 level.

**Significant stimulation of superoxide anion production at P<0.01 level.

Table 4. Effects of Glucan on Nitrite Oxide Production

| Glucan | Nitrite oxide | (μmol/L) | |
|-------------------|-------------------|-------------|-------------|
| | Mouse neutrophils | HL-60 | U937 |
| Maitake Gold | 4.11 ± 0.11 | 3.98 ± 0.23 | 5.79 ± 0.43 |
| Krestin | 5.27 ± 0.78 | 4.98 ± 0.66 | 5.56 ± 1.11 |
| Aktival | 5.04 ± 0.57 | 3.87 ± 0.87 | 5.52 ± 0.54 |
| Immutol | 2.99 ± 0.77 | 3.68 ± 0.54 | 4.61 ± 0.99 |
| Now Glucan | 5.99 ± 0.68 | 6.01 ± 1.12 | 7.21 ± 1.07 |
| Sweet Beta Glucan | 2.88 ± 0.44 | 4.01 ± 0.95 | 4.24 ± 1.01 |
| Beta Right | 4.61 ± 0.87 | 5.55 ± 0.99 | 6.12 ± 1.22 |
| Epicor | 4.33 ± 1.54 | 3.75 ± 0.89 | 4.87 ± 0.98 |
| NSC | 3.78 ± 0.66 | 3.86 ± 0.75 | 4.44 ± 1.04 |
| Glucagel | 6.46 ± 1.06 | 5.15 ± 0.78 | 6.69 ± 1.25 |
| Better Immunity | 2.98 ± 1.43 | 3.78 ± 1.01 | 4.99 ± 0.98 |
| Macroforce | 3.56 ± 0.55 | 4.72 ± 0.87 | 5.01 ± 0.99 |
| Solgar | 4.55 ± 1.07 | 4.88 ± 1.01 | 5.11 ± 1.27 |
| Wellmune | 7.01 ± 1.77 | 5.36 ± 0.79 | 6.98 ± 1.21 |
| Swanson | 4.22 ± 0.99 | 4.87 ± 2.02 | 4.98 ± 1.78 |
| Glucan #300 | 7.87 ± 1.55 | 6.26 ± 1.77 | 8.37 ± 2.03 |
| PBS | 0.24 ± 0.04 | 0.09 ± 0.02 | 0.31 ± 0.05 |

Stimulation of superoxide anion production was significant at P<0.01 level.

Table 5. Effect of Glucan on Secretion of IL-2

| Glucan | IL-2 (pg/ml) |
|-------------------|-----------------|
| Maitake Gold | 695.7± 23.9 |
| Krestin | 601.2 ± 109.5 |
| Aktival | 445.7 ± 95.7 |
| Immutol | 326.5 ± 99.6 |
| Now Glucan | 525.6 ± 109.5 |
| Sweet Beta Glucan | 237.3 ± 67.4 |
| Beta Right | 678.1± 156.3 |
| Epicor | 113.8 ± 27.8 |
| NSC | 267.1 ± 50.5 |
| Glucagel | 206.7 ± 56.4 |
| Better Immunity | 156.0 ± 33.9 |
| Macroforce | 358.9 ± 87.1 |
| Solgar | 89.9 ± 33.4 |
| Wellmune | 711.2 ± 167.5 |
| Swanson | 177.8 ± 36.6 |
| Glucan #300 | 983.9 ± 122.8 |
| Con A | 1 047.6 ± 287.7 |

All glucans showed significant stimulation of IL-2 secretion at P<0.01 level.

Table 6. Effects of Glucans on Formation of Antibodies Against Ovalbumin

| Glucan | % of control |
|-------------------|---------------|
| Maitake Gold | 211.7± 20.1* |
| Krestin | 181.3 ± 39.1 |
| Aktival | 85.3 ± 17.3 |
| Immutol | 136.2 ± 33.0 |
| Now Glucan | 215.4 ± 50.1* |
| Sweet Beta Glucan | 33.6 ± 7.2 |
| Beta Right | 278.9± 43.7* |
| Epicor | 38.1 ± 7.0 |
| NSC | 137.7 ± 20.8 |
| Glucagel | 396.5 ± 50.2* |
| Better Immunity | 247.1 ± 29.5* |
| Macroforce | 143.8 ± 25.3 |
| Solgar | 55.6 ± 13.0 |
| Wellmune | 322.6 ± 47.2* |
| Swanson | 111.4 ± 14.4 |
| Glucan #300 | 343.9 ± 43.1* |
| Ovalbumin | 164.2 ± 28.5 |
| Ovalbumin + FA | 493.8 ± 57.4* |

FA – Freud's adjuvant.

*Significant at <0.05 level.

focused either on the description of newly isolated glucans or on the description of their biological activities. Comprehensive reviews comparing several glucans are rare. In one of those, Yadomae reviewed how structural properties of glucans affected biological activities and found that branched or linear 1,4 glucans have limited activity and β glucans with a 1,3 configuration with additional branching at the position 0-6 of the 1-3 linked D-glucose residues have the highest immunostimulating activity [15]. Readers seeking additional reviews might look up Kogan [16] or Vetvicka [17]. However, it is important to keep in mind that these reviews are oriented towards comparing results of numerous publications and none of them offers a one-on-one comparison of several glucans. Very few studies tried to directly compare individual glucans and the number of used glucans was seriously limited [5, 7-8].

Glucans are well known to stimulate cellular immunity, thus phagocytosis is one of the first tests of the immunological characteristics of any glucan. We used the 2-hydroxyethyl methacrylate particles that have only a slight negative charge and as a result do not nonspecifically adhere to the cell surface [18]. This guarantees that only phagocytosing cells will engulf these particles and significantly lowers the chance of false negativity. Our investigation showed that while most of the tested glucans to some extent stimulated phagocytosis of synthetic microspheres, these effects were usually nonsignificant or achieved only using the highest dose.

Ligand-receptor interactions during phagocytosis result in a substantial outburst of metabolic activity. During these initial stages of endocytosis, the phagocytes exhibited a large increase in oxygen consumption, hexosemonophosphate shunt activity, and production of active oxygen species. This process is known as the oxidative burst. Production of active oxygen molecules is necessary for the destruction of invading microorganism. As several glucans have been shown in past to stimulate oxidative burst [19-21], we evaluated our glucan on stimulation of superoxide anion and nitrite oxide.

In addition to the direct effect on various cells of the immune system, the immunostimulating action of β -glucans is caused by potentiation of a synthesis and release of several cytokines such as TNF α , IFN γ , IL-1 and IL-2. This cytokine stimulating activity is thought to be dependent on the triple helix conformation [22]. The only glucan without a trace of pro-inflammatory cytokine stimulation is PGG-glucan [23]. We focused on the stimulation of IL-2 production by spleen cells *in vitro* and found that whereas all glucans significantly stimulated production of IL-2, only one of the samples (Glucan #300) showed stimulation comparable to the common stimulator Concanavalin A. The activity of the most active glucan was comparable to the previously published data [6, 13].

Glucans are usually considered stimulators of the cellular branch of immune reaction and only limited attention has been focused on their potential effects on antibody response. However, recent studies showed that glucan not only potentiated antibody response [24], but can be used for robust stimulation of both cellular and humoral immune responses during vaccination [25]. Rather surprisingly, our results showed that all of the tested glucans revealed some level of stimulation of antibody response, the strongest being Glu-

cagel T and Glucan #300. However, the stimulation was always significantly lower than in the case of combining antigen and Freund's adjuvant.

Data presented in this study clearly demonstrated significant differences in activities among individual types of glucans. In addition, it is clear that individual glucans can be highly active in one particular part of immune reaction (e.g., Glucagel T on antibody production), and mediocre in other parts of immune reaction. Glucan #300 showed the highest activity in all tested reactions (with the exception of the antibody formation where it was close second). Some of the tested glucans (Beta Right 101, Swanson and Glucan #300) case from the same manufacturer, but still strongly differed in their immunostimulating activities. The most probable explanation is the purity of these glucans – Glucan #300 has purity over 85%, whereas Beta Right 101 and Swanson have purity of app. 70 percent.

CONCLUSION

Several conclusions can be made: 1) not all glucans are created equal; 2) some of the commercial glucans have surprisingly low activity; 3) most glucans strongly differ in biological effects based on tested characteristics, and 4) no clear relevance between the source of glucan and its activity has been found. Also, another important point is the fact that some of the glucans exhibited low activity. In these cases, however, you need up to 100x more of glucan to illicit high activity. This means that the low activity is not caused by a lower percentage of glucan but, rather, that the glucans with limited biological activities will not be comparable to the “better” glucans regardless of the dosage.

CONFLICT OF INTEREST

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SUPPLEMENTARY MATERIAL

Supplementary material is available on the publishers Web site along with the published article.

REFERENCES

- [1] Benacerraf B, Sebastyen MM. Effect of bacterial endotoxins on the reticuloendothelial system. *Fed Proc* 1957; 16: 860-7.
- [2] Rigi SJ, Di Luzio NR. Identification of a reticuloendothelial stimulating agent in zymosan. *Am J Physiol* 1961; 200: 297-300.
- [3] Novak M, Vetvicka V. Beta-glucans, history and the present: Immunomodulatory aspects and mechanisms of action. *J Immunotoxicol* 2008; 5: 47-57.
- [4] Novak M, Vetvicka V. Glucans as biological response modifiers. *Endocrine Metabol Immune Disorders-Drug Targets* 2009; 9: 67-75.
- [5] Vetvicka V, Vetvickova J. A comparison of injected and orally administered beta glucans. *J American Nutr Assoc* 2008; 11: 42-8.
- [6] Vetvicka V, Vetvickova J. Immunostimulating properties of two different β -glucans isolated from Maitake mushroom (*Grifola frondosa*). *J Am Nutr Assoc* 2005; 8: 33-9.
- [7] Vetvicka V, Vetvickova J. An evaluation of the immunological activities of commercially available β 1,3-glucans. *J Am Nutr Assoc* 2007 a; 10: 25-31.
- [8] Vetvicka V, Vetvickova J. Physiological effects of different types of β -glucan. *Biomed Pap Med Fac Univ Palacky* 2007b; 151: 225-31.
- [9] Vetvicka V, Fornusek L, Kopecek J, Kaminkova J, Kasperek L, Vranova M. Phagocytosis of human blood leukocytes: A simple micromethod. *Immunol Lett* 1982; 5: 97-100.
- [10] Vetvicka V, Holub M, Kovaru H, Siman P, Kovaru F. Alpha-fetoprotein and phagocytosis in athymic nude mice. *Immunol Lett* 1988; 19: 95-8.
- [11] Fernandez-Botran R, Vetvicka V. *Advanced Methods in Cellular Immunology*, CRC Press, Boca Raton 2000.
- [12] Green SJ, Nacy CA. Antimicrobial and immunopathological effects of cytokine-induced nitric oxide synthesis. *Curr Opin Infect Dis* 1993; 6: 284-396.
- [13] Vetvicka V, Terayama K, Mandeville R, Brousseau P, Kournikakis B, Ostroff G. Pilot study: orally administered yeast β 1,3-glucan prophylactically protects against anthrax infection and cancer in mice. *J Am Nutr Assoc* 2002; 5: 1-5.
- [14] Vetvicka V, Yvin J-C. Effects of marine β -glucan on immune reaction. *Int Immunopharmacol* 2004; 4: 721-30.
- [15] Yadomae T. Structure and biological activities of fungal β 1,3-glucans. *Yakugaku Zasshi* 2000; 120: 413-31.
- [16] Kogan G. In: A. Atta-ur-Rahman, Eds. *Studies in natural products chemistry*. Amsterdam: Elsevier 2000.
- [17] Vetvicka V. β Glucans as immunomodulators. *J Am Nutr Assoc* 2001; 3: 31-4.
- [18] Vetvicka V, Fornusek L. Polymer microbeads in immunology. *Biomaterials* 1987; 8: 341-5.
- [19] Wakshull E, Brunke-Reese D, Linderthum J, *et al*. PGG-glucan, a soluble β -(1,3)-glucan, enhances the oxidative burst response, microbicidal activity, and activates an NK- κ B-like factor in human PMN: Evidence for a glycosphingolipid β -(1,3)-glucan receptor. *Immunopharmacology* 1999; 41: 89-107.
- [20] Menard R, Ruffray P, Fritig B, Yvin J-C, Kaufmann S. Defence and resistance-inducing activities in tobacco of the sulfated beta-1,3 glucan PS3 and its synergistic activities with the unsulfated molecule. *Plant Cell Physiol* 2005; 46: 1964-72.
- [21] Nerren JR, Kogut MH. The selective Dectin-1 agonist, curdlan, induces an oxidative burst response in chicken heterophils and peripheral blood mononuclear cells. *Vet Immunol Immunopathol* 2009; 127: 162-6.
- [22] Falch BH, Espevik T, Ryan L, Stokke BT. The cytokine stimulating activity of (1-3)- β -D glucans is dependent on the triple helix conformation. *Carbohydr Res* 2000; 329: 587-96.
- [23] Bleicher P, Mackin W. Betafectin PGG-Glucan: A novel carbohydrate immunomodulator with anti-infective properties. *J Biotechnol Healthcare* 1995; 2: 207-22.
- [24] Vetvicka V, Dvorak B, Vetvickova J, *et al*. Orally-administered marine β 1,3 glucan Phycarine stimulates both humoral and cellular immunity. *Int J Biol Macromol* 2007; 40: 291-8.
- [25] Huang H, Ostroff GR, Lee CK, Specht CA, Levitz SM. Robust stimulation of humoral and cellular immune responses following vaccination with antigen-loaded β -glucan particles. *mBio* 1, doi:10.1128/mBio.00164-10
- [26] Hong F, Yan J, Baran JT, *et al*. Mechanism by which orally-administered beta-1,3-glucans enhance the tumoricidal activity of antitumor monoclonal antibodies in murine tumor models. *J Immunol* 2004; 173: 797-806.
- [27] Hunter KW, duPre S, Redelman D. Microparticulate B-glucan upregulates the expression of B7.1, B7.2, B7-H1. but not B7-DC on cultured murine peritoneal macrophages. *Immunol Lett* 2004; 93: 71-8.
- [28] Weitberg AB. A phase I/II of beta-(1,3)/(1,6) D-glucan in the treatment of patients with advanced malignancies receiving chemotherapy. *J Exp Clin Cancer Res* 2008; 27: 40-4.

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SUPPLEMENTARY MATERIAL

It is important to note that some of the tested glucans (namely Beta Right, Better Immunity, Swanson, Wellmune and Glucan #300) are manufactured by the same company, but still show significant differences in their ability to stimulate immune system. This discrepancy is most probably caused by different purity among these particular glucans, which range from over 85% in case of Glucan #300 to app. 70% in others.

Table 1. Types of Glucan Used in this Study

| Glucan | Source | Solubility | Manufacturer |
|----------------------|---------------|-------------------|---|
| BetaMax | Yeast | Insoluble | Chisolm Biological Laboratores, Aiken, SC |
| Oat Beta Glucan | Oat | Insoluble | Health Breakthroughs, Lake Oswego, OR |
| Beta 1,3 Glucan | Yeast | Insoluble | Viramin World, Ronkonkoma, NY |
| Imuneks | Yeast | Insoluble | Mustafa Nevzat, Istanbul, Turkey |
| Bio-Glucan | Yeast | Insoluble | Pharma Nord, Vojens, Denmark |
| Beta Glucan | Yeast | Insoluble | Greenpath, Wrightsville Beach, NC |
| Qore Defense | Mushroom | Insoluble | Quivana, Provo, UT |
| Beta Glucan | Yeast | Insoluble | Our Health Co-op, Riviera Beach, FL |
| ImmunotiX 3,6 | Yeast | Insoluble | Xymogen, Orlando, FL |
| Hliva ustricna | Mushroom | Insoluble | Terezia Company, Prague, Czech Republic |
| Betacan 500 | Yeast | Insoluble | Arrowhead Healthworks, Cedarpines Park, CA |
| GlucanReal | Mushrooms | Soluble | QueGen Biotech, South Korea |
| GlucanPure | Mushrooms | Soluble | QueGen Biotech, South Korea |
| Hliva ustricna | Mushrooms | Insoluble | Walmart, Trinec, Czech Republic |
| Beta Glucan | Yeast | Insoluble | Source Naturals, Scotts Valley, CA |
| MC-Glucan | Mushroom | Soluble | IntaPort, East Brunswick, NJ |
| MC-Glucan | Mushroom | Soluble | Macrocare Tech, South Korea |
| Beta Glucan | Yeast | Insoluble | Biotikon, Germany |
| Immune Builder | Mushrooms | Insoluble | MushroomScience, Eugene, OR |
| Krestin | Mushrooms | Soluble | Kureha Chemical, Tokyo, Japan |
| Senseiro Liquid Gold | Mushrooms | Soluble | Senseiro, Osaka, Japan |
| Glucan | Mushrooms | Soluble | Ajinomoto, Tokyo, Japan |
| Beta Glucan | Mushrooms | Soluble | 7Red, Ostrava, Czech Republic |
| Glucan Komplex | Yeast | Insoluble | NaturProdukt, Czech Republic |
| Mushroom Glucan | Mushroom | Soluble | Perfect Health Biotechnology, SanChung City, Taiwan |
| Hliva ustricna | Mushroom | Insoluble | Jedlehouby, Dolany, Czech Republic |
| Phycarine | Seaweed | Soluble | Goemar, St. Malo, France |
| PS3 | Seaweed | Soluble | Goemar, St. Malo, France |
| Pleuran | Mushrooms | Insoluble | Pleuran, Bratislava, Slovakia |
| Immunoglucan | Yeast | Insoluble | Hankitatikku, Kakkila, Finland |
| Glucan | Yeast | Insoluble | Sigma, St. Louis, MO |
| Glucan | Barley | Semisoluble | Sigma, St. Louis, MO |