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Research Article

Different β-Glucans – Different Effects On Natural, Adaptive And Trained Immunity.

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Abstract

β-Glucans are among the best-characterized natural immunomodulators and are isolated from a wide range of sources, including yeast, mushrooms, cereals, and algae. Despite extensive worldwide research, it remains unclear which β-glucan preparations exhibit the greatest immunomodulatory efficacy. In the present study, we compared the effects of several commercially available β-glucans on innate, adaptive, and trained immune responses.

Keywords: β-Glucan; phagocytosis; IL-2; antibody; trained immunity.

INTRODUCTION

The immune system is traditionally divided into two major components: innate (natural) and adaptive (acquired) immunity. Innate immunity is a non-specific defense system that is present at birth. It responds rapidly to invading pathogens but does not retain immunological memory. In contrast, adaptive immunity is a highly specialized system that develops throughout life following exposure to infectious agents. Although adaptive immune responses require more time to develop, they generate memory cells that enable a faster and more effective response upon subsequent exposure to the same pathogen.

During the last decade, a third concept of immune defense, termed trained immunity, has emerged.[1] Trained immunity refers to a long-term functional reprogramming of innate immune cells through epigenetic and metabolic modifications, resulting in an altered response to a subsequent, unrelated challenge.[2,3]

β-Glucan is the most extensively studied biological response modifier and has been shown to exert potent stimulatory effects on multiple components of the immune system. Despite more than 55,000 published studies, the reported biological activities of β-glucans remain inconsistent. This variability is largely attributable to structural differences among individual glucans, including molecular weight, branching pattern, solubility, and source, all of which influence their biological activity.[4] Furthermore, the limited number of direct comparative studies has demonstrated substantial differences in the immunomodulatory activities of individual glucan preparations.[5,6]

MATERIALS AND METHODS

Animals

Female BALB/c mice (8 weeks of age) were purchased from The Jackson Laboratory (Bar Harbor, ME, USA). All animal experiments were conducted in accordance with the Institutional Animal Care and Use Committee (IACUC) guidelines of the University of Louisville. Mice were euthanized by CO_2 asphyxiation followed by cervical dislocation.

Cell line

The BALB/c mouse-derived mammary tumor cell line Ptas64 was generously provided by Dr. Wei-Zen Wei (Michigan Cancer Foundation, Wayne State University, Detroit, MI, USA). Cells were maintained in RPMI 1640 medium containing HEPES buffer and supplemented with 10% heat-inactivated fetal calf serum (FCS), 100 U/mL penicillin, and 100 µg/mL streptomycin. Cultures were maintained in tissue culture flasks at 37 °C in a humidified incubator containing 5% $CO_2$and 95% air.

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Materials

All β-glucan samples were either donated by the manufacturers or purchased from their distributors, as listed in **Table 1**. Glucans were tested as sold at retail, dosed by equal product mass, without independent verification of content. Ovalbumin, polymyxin B, cyclophosphamide, and Wright stain were purchased from Sigma (St. Louis, MO, USA).

Table 1. Individual glucans.

Glucan	Source	Manufacturer
5 Defenders	mushroom	Real Mushrooms, Calgary, Canada
Turkey Tail	mushroom	Eos Corp., Hong Kong, China
Host Defense Mushroom	mushroom	Fungi Perfecti, Olympia, WA, USA
Maitake D	mushroom	Mushroom Wisdom, Rutherford, NJ, USA
RYL Beta 550	yeast	Youngevity, Scottsdale, AZ, USA
ABBi16	yeast	AB Biotek, Casteggio, Italy
#300	yeast	Transfer Point, Columbia, SC, USA

Phagocytosis assay

Phagocytosis of 2-hydroxyethyl methacrylate (HEMA) microspheres was performed as previously described.7 Briefly, 0.1 mL of peripheral blood collected from mice treated with various doses of β-glucan or phosphate-buffered saline (PBS) was incubated in vitro with 0.05 mL of HEMA microspheres (5×10^8 particles/mL) at 37 °C for 60 min with intermittent shaking. Blood smears were prepared and stained with Wright stain. Cells containing three or more HEMA particles were considered phagocytosis-positive. All experiments were performed in triplicate, and at least 300 cells were evaluated per sample.

IL-2 secretion

Purified splenocytes (2×10^6 cells/mL) isolated from mice treated with 100 µg of β-glucan or PBS were suspended in RPMI 1640 medium supplemented with 5% FCS and seeded into 24-well tissue culture plates. Cells were incubated for 48 h at 37 °C in a humidified atmosphere containing 5% CO_2. Concanavalin A (1 µg/well; Sigma) served as the positive control. At the end of the incubation period, culture supernatants were collected, and IL-2 concentrations were determined using a Quantikine Mouse IL-2 ELISA kit (Abcam, Cambridge, UK).

Antibody response

The antibody response to ovalbumin was evaluated as previously described.5 Mice were immunized twice, 14 days apart, with 100 µg of ovalbumin. Blood samples were collected on Day 21, and serum was isolated for analysis. Experimental groups received daily intraperitoneal (i.p.) injections of β-glucan throughout the experimental period. Ovalbumin-specific antibody levels were determined by ELISA. Mice immunized with ovalbumin emulsified in Freund's adjuvant (Sigma) served as the positive control.

Trained immunity

To evaluate the induction of trained immunity, mice received a single intraperitoneal injection of 100 µg of the indicated β-glucan preparation. Seven days later, animals were inoculated orthotopically into the mammary fat pad with 1×10^6 Ptas64 cells suspended in PBS. Three weeks after tumor implantation, mice were euthanized, tumors were excised, and tumor weights were determined as previously described.[8]

RESULTS

Commercial dietary supplements contain small amounts of biologically inert excipients that are not expected to possess immunological activity. Nevertheless, it is theoretically possible that unknown quantities of these excipients could affect the actual amount of active ingredient present in a capsule. For example, a capsule labeled as containing 10 mg of β-glucan may have a total weight exceeding 10 mg because of the presence of inactive fillers. To address this possibility, we previously compared the biological activity of a β-glucan preparation available both as a purified powder and as encapsulated capsules. The activities of equivalent doses prepared from both formulations were identical, even at the lowest dose tested.[7]

We first evaluated phagocytosis, one of the primary effector functions of innate immunity. Phagocytosis is the process by which phagocytic cells engulf and subsequently digest microorganisms, foreign particles, and cellular debris. In order to find the optimal dose, we evaluated the effets of three different doses. β-Glucans enhance phagocytic activity through interactions with several pattern-recognition receptors, particularly complement receptor 3 (CR3) and Dectin-1.

Phagocytic activity was assessed using synthetic 2-hydroxyethyl methacrylate (HEMA) microspheres following two weeks of treatment with three different doses of each

β-glucan preparation. As summarized in **Table 2**, most β-glucan samples significantly enhanced phagocytosis in a dose-dependent manner. The greatest stimulatory activity was observed with Glucan #300.

Table 2. Effects of various glucans on phagocytosis.

Sample	50 (μg)	100 (μg)	200 (μg)
5 Defenders	38.4 ± 1.2*	40.5 ± 2.2*	42.8 ± 3.1*
Turkey Tail	32.8 ± 2.1	36.2 ± 2.0	37.9 ± 1.5*
Host Defense Mushroom	38.0 ± 2.9*	44.1 ± 3.3*	48.1 ± 3.7*
Maitake D	38.8 ± 2.2*	44.8 ± 1.8*	52.1 ± 3.7*
RYL Beta 550	40.2 ± 1.9*	46.6 ± 3.0*	48.9 ± 2.6*
ABBi16	43.5 ± 2.5*	46.6 ± 3.2*	50.1 ± 3.8*
#300	47.2 ± 2.9*	58.6 ± 4.0*	67.8 ± 3.9*

*Control values (PBS) were 31.9 ± 2.9. The dose means a single ip. injection in PBS/mouse. *Significant difference between tested groups and PBS control group at P ≤ 0.05 level. Results represent mean values from three experiments ± SD.*

We next examined IL-2 production by splenocytes isolated from control and β-glucan-treated mice. Following 72 h of in vitro incubation, IL-2 concentrations were measured in culture supernatants. Because unstimulated spleen cells produced no detectable IL-2, all β-glucan preparations significantly increased IL-2 secretion (**Table 3**). Once again, Glucan #300 exhibited the highest activity. However, none of the tested preparations induced IL-2 production to the level achieved by the Concanavalin A positive control.

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

Sample	100 (μg)
5 Defenders	181.5 ± 11.2*
Turkey Tail	119.3 ± 18.6*
Host Defense Mushroom	308.4 ± 21.8*
Maitake D	380.4 ± 25.5*
RYL Beta 550	155.2 ± 12.9*
ABBi16	503.9 ± 21.8*
#300	874.4 ± 32.1*
Neg. Control	0
Pos. Control	994.8 ± 35.7*

**Significant difference between tested groups and PBS control group at P ≤ 0.05 level. Results represent mean values from three experiments ± SD.*

The effects of β-glucan treatment on adaptive immunity were further evaluated by measuring the antibody response to ovalbumin. Mice were immunized twice with ovalbumin at two-week intervals, and serum samples were collected seven days after the second immunization. Most β-glucan preparations significantly enhanced antigen-specific antibody production (**Table 4**). The strongest responses were observed in mice treated with Maitake D and Glucan #300, whereas the Host Defense Mushroom preparation did not produce a statistically significant effect.

Table 4. Effect of glucan supplementation on antibody formation.

Glucan	% of control (ovalbumin only)
5 Defenders	189.0 ± 14.9*
Turkey Tail	208.9 ± 19.7*
Host Defense Mushroom	111.6 ± 15.2
Maitake D	300.4 ± 34.7*
RYL Beta 550	153.5 ± 17.9*
ABBi16	253.3 ± 20.1*
#300	344.1 ± 24.1*
Ovalbumin + FA	461.7 ± 30.8*

**Significant difference between tested groups and PBS control group at P ≤ 0.05 level. Results represent mean values from three experiments ± SD.*

Finally, we investigated the ability of β-glucans to induce trained immunity capable of suppressing breast tumor growth. Mice received a single administration of the test samples prior to tumor challenge. As shown in **Table 5**, pretreatment with Turkey Tail, Maitake D, and Glucan #300 significantly inhibited subsequent breast tumor growth, demonstrating their ability to induce trained immunity with measurable antitumor activity.

Table 5. Effets of glucan on tumor growth.

Glucan	mg of tumor weight
5 Defender	602.1 ± 51.1
Turkey Tail	484.6 ± 28.4*
Host Defense Mushroom	625.4 ± 49.3
Maitake D	508.6 ± 37.7*
RYL Beta 550	671.1 ± 30.9
ABBi16	638.4 ± 40.1
#300	348.9 ± 27.3*
PBS	725.5 ± 38.9

**Significant difference between tested groups and PBS control group at P ≤ 0.05 level. Results represent mean values from three experiments ± SD.*

DISCUSSION

The immunomodulatory activity of β-glucans is determined by multiple structural characteristics rather than by a single property. The glucan preparations that consistently demonstrated strong activity across phagocytosis, IL-2 production, antibody formation, and trained immunity most likely possess an optimal combination of favorable structural features, whereas the less active preparations differ in one or more of these characteristics. The principal determinants of β-glucan biological activity are discussed below.

Yeast-derived β-glucans from *Saccharomyces cerevisiae* consist of a β-(1,3)-linked backbone with β-(1,6)-linked side chains, a structural configuration that is efficiently recognized by innate immune receptors. In contrast, mushroom-derived β-glucans vary considerably among species, and many commercial mushroom preparations are produced from mycelia cultivated on grain substrates, resulting in relatively low and highly variable β-glucan content. Cereal β-glucans are composed primarily of mixed β-(1,3)/(1,4) linkages and interact only weakly with Dectin-1. Moreover, even among S. cerevisiae preparations, differences in yeast strain and cultivation conditions can substantially influence branching pattern, molecular weight, purity, and overall biological activity. Consequently, two products marketed simply as "yeast β-glucan" may exhibit markedly different immunomodulatory properties.

Molecular weight is another major determinant of biological activity. In general, high-molecular-weight β-glucans are more potent immunomodulators because they activate complement more efficiently and are capable of inducing trained immunity. In contrast, low-molecular-weight fragments and short β-glucan oligosaccharides generally display reduced biological activity. Thus, molecular weight likely contributes substantially to the differences in activity observed among the preparations evaluated in the present study.

The physical form of β-glucan also influences its biological effects. Particulate β-glucans, including whole-glucan particles, promote clustering of Dectin-1 receptors at the cell surface, forming a phagocytic synapse that enhances phagocytosis, oxidative burst, and cytokine production. These particulate forms are also those most consistently associated with the induction of trained immunity. In contrast, soluble β-glucans preferentially interact with complement receptor 3 (CR3) and generally induce a different, and often less pronounced, cytokine profile. The coordinated enhancement of phagocytosis, IL-2 production, and trained immunity observed for the most active preparations in the present study is consistent with the activity of intact, high-molecular-weight particulate β-glucans acting primarily through Dectin-1-mediated signaling.[9]

Trained immunity enhances host resistance by functionally reprogramming innate immune cells through long-lasting epigenetic and metabolic modifications. Macrophages and monocytes are the principal cellular targets of this process. β-Glucan-induced trained immunity has been shown to enhance antibody responses and improve vaccine efficacy, including experimental cancer vaccines. Notably, a single administration of β-glucan is sufficient to induce these long-lasting functional changes.[10] Consequently, β-glucans are increasingly recognized as promising inducers of trained immunity and have attracted considerable interest as potential adjuvants in cancer immunotherapy.[11]

The present study demonstrated substantial differences in the biological activities of commercially available β-glucan preparations. Among the products evaluated, Glucan #300 consistently exhibited the greatest immunostimulatory

activity across multiple assays. Several additional preparations displayed moderate immunological activity, whereas others produced only limited stimulation of immune function. Whether yeast-derived β-glucans are intrinsically superior to mushroom-derived β-glucans remains uncertain, as biological activity depends not only on the source but also on molecular weight, branching structure, purity, solubility, particle size, and manufacturing procedures. Further studies directly comparing well-characterized β-glucan preparations will be necessary to define the structural features responsible for optimal immunomodulatory activity.

REFERENCES

1. Netea, M.G., Joosten, L.A., Latz, E., Mills, K.H., Natoli, G., Stunnenberg, H.G., O'Neill, L.A., Xavier, R.J. 2016. Trained immunity: A program of innate immune memory in health and disease. Science, 352. doi: 10.1126/science.aaf1098. PMID: 27102489; PMCID: PMC5087274.

2. Netea, M.G., Quintin, J., van der Meer, J.W. 2011. Trained immunity: a memory for innate host defense. Cell Host Microbe, 9:355. doi: 10.1016/j.chom.2011.04.006.

3. Vuscan, P., Kischkel, B., Hatzioannou, A., Markaki, E., Sariea, A., Tintore, M., Curie, J., Verginis, P., de Kecea, C., Chavakis, T., Joosten, L.A.B., Netea, M.G. 2024. Potent induction of trained immunity by Saccharomyces cerevisiae β-glucans. Frontiers Immunol., doi:10.3389/fimmu.2024.1323333.

4. Zhong, X., Wang, G., Li, F., Fang, S., Zhou, S., Ishiwata, A., Tonevitsky, A.G., Shkurnikov, M., Cai, H., Ding, F. 2023. Immunomodulatory effect and biological significance of β-glucans. Pharmaceutics, 15: 1615. doi: 10.3390/pharmaceutics15061615

5. Vetvicka, V., Vetvickova, J. 2010. Beta 1,3-glucan: Silver bullet or hot air? Open Glycoscience 3:1-6.

6. Vetvicka, V., Vetvickova, J. 2014. Comparison of immunological effects of commercially available beta-glucans. Appl. Sci. Rep. 1:1-7.

7. Vetvicka, V., Vetvickova, J. 2018. Glucans and cancer: Comparison of commercially available β-glucans - Part IV. Anticancer Res. 38:1327-1333. doi: 10.21873/anticanres.12355.

8. Vetvicka, V., Yvin, J-C. 2004. Effects of marine β-1,3 glucan on immune reactions. Int. Immunopharmacol., 4: 721-730.

9. Elder, M.J., Webster, S.J., Chee, R., Williams, D.L., Gaston, J.S., Goodall, J.C. 2017. β-Glucan size controls dectin-1-mediated immune responses in human dendritic cells by regulating IL-1β production. Front. Immunol., 8:791. doi: 10.3389/fimmu.2017.00791

10. Ajit, J., Chen, Q., Ung, T., Rosenberger, M., Kim, J., Solanki, A., Shen, J., Esser Kahn, A.P. 2025. β-Glucan induced trained immunity enhances antibody levels in a vaccination model in mice. PLoS One. 20:e0323376. doi: 10.1371/journal.pone.0323376.

11. Sui, Y, Berzofsky, J.A. 2024. Trained immunity inducers in cancer immunotherapy. Front. Immunol. 15:1427443. doi: 10.3389/fimmu.2024.1427443