

IN THIS EDITION

- Pilot Study: Orally-Administered Yeast β 1,3-glucan Prophylactically Protects Against Anthrax Infection and **Cancer in Mice**
- Results of a Study Evaluating the Use of a Dietary Supplement Formula in the Management of Age-Related Skin Changes in Women with Moderate to Severe Wrinkling of the Periorbital Area
- St. John's Wort for Depression: Weight of All the Evidence Still Favors Effectiveness of Herb for Mild to Moderate Depressive Disorders
- The Potential Application of *Spirulina (Arthrospira)* as a Nutritional and Therapeutic Supplement in Health Management

AND MORE



A Peer-Reviewed Journal on Nutraceuticals and Nutrition

ISSN-1521-4524



**Journal of the
American
Nutraceutical
Association**

EDITORIAL STAFF

EDITOR-IN-CHIEF
Mark Houston, MD

EDITORS
Medicine - Christopher M. Foley, MD
Pharmacy - Allen M. Kratz, PharmD

ASSOCIATE EDITORS
Bernd Wollschlaeger, MD
Lisa Colodny, PharmD

TECHNICAL EDITOR
Jane Lael

ART DIRECTOR
Gary Bostany

EDITORIAL BOARD
Jan Basile, MD
Russell Blaylock, MD
Jerome B. Block, MD
Lisa Colodny, PharmD, BCNSP
Loren Cordain, PhD
Derrick DeSilva, MD
Jeanette Dunn, EdD, RN, CNS
Brent Eagan, MD
Natalie D. Eddington, PhD
Clare M. Hasler, PhD
Ralph Hawkins, MD
Mark C. Houston, MD, FACP
David S. Hungerford, MD
Robert Krueger, PhD
Alexander Mauskop, MD
Mark J.S. Miller, PhD
June Reidlinger, PharmD, RPh
Anthony J. Silvagni, DO, PharmD, MSc
John Strupp, MD
C. Wayne Vewart, PharmD, BCPS, FASHP
Farred Wassef, RPh
Bernd Wollschlaeger, MD

American Nutraceutical Association
Executive Office
5120 Selkirk Drive, Suite 100
Birmingham, AL 35242
Phone: (205) 980-5710 Fax: (205) 991-9302
Website: www.Ana-Jana.org

CEO & PUBLISHER
Allen Montgomery, RPh

ANA is an alliance of individuals with interest in nutraceutical science, technology, marketing and production. It was established to develop and provide educational materials and continuing education programs for health care professionals on nutraceutical technology and science. ANA publishes a monthly E-newsletter, *The Grapevine*, and the *Journal of the American Nutraceutical Association (JANA)*.

The Journal of the American Nutraceutical Association (ISSN-1521-4524) is published four times annually by the American Nutraceutical Association (ANA). Send all inquiries, letters, and submissions to the ANA Editorial Department at 5120 Selkirk Drive, Suite 100, Birmingham, AL 35242. Contents © 2002 ANA, all rights reserved. Printed in the United States of America. Reproduction in whole or part is not permitted without written permission. It is the responsibility of every practitioner to evaluate the appropriateness of a particular opinion in the context of actual clinical situations. Authors, editors, and the publisher cannot be held responsible for any typographical or other errors found in this journal. Neither the editors nor the publisher assume responsibility for the opinions expressed by the authors.

Cover Photograph - Sierra Productions, Irvine, CA.

Contents – Spring 2002, Vol. 5, No. 2

GUEST EDITORIAL

St. John's Wort for Depression: Weight of All the Evidence Still Favors Effectiveness of Herb for Mild to Moderate Depressive Disorders 1
R. William Soller, PhD

EDITORIAL COMMENTARY

Yeast β 1,3-glucan and Its Use Against Anthrax Infection and in the Treatment of Cancer 3
Russell L. Blaylock, MD

ORIGINAL RESEARCH

Pilot Study: Orally-Administered Yeast β 1,3-glucan Prophylactically Protects Against Anthrax Infection and Cancer in Mice 5
Vaclav Vetvicka, PhD, Kiyomi Terayama, MD, Rosemonde Mandeville, MD, PhD, Pauline Brousseau, PhD, Bill Kournikakis, PhD, Gary Ostroff, PhD

Results of a Study Evaluating the Use of a Dietary Supplement Formula in the Management of Age-Related Skin Changes in Women with Moderate to Severe Wrinkling of the Periorbital Area 10
Irwin Kantor, MD, FAAD, Louise A. Donikyan, DO, Randi Simon, BS, Bernd Wollschlaeger, MD

Efficacy and Tolerance of an Ephedra-Free Nutraceutical Weight Management Product in an Asian Population 20
Paul Bobrowski, BS, Cynthia Chan, BS, Kim Ho, BS, Mark J.S. Miller, PhD

REVIEW ARTICLE

The Potential Application of *Spirulina (Arthrospira)* as a Nutritional and Therapeutic Supplement in Health Management 27
Amha Belay, PhD

To subscribe to JANA phone 800-566-3622,
outside USA 205-833-1750.

To order reprints of articles, or additional copies of JANA,
contact:

Deana Hunter, Public Relations Director
5120 Selkirk Drive, Suite 100, Birmingham, AL 35242
Phone 205-980-5710 Fax 205-991-9302
E-mail: DeanaH@ana-jana.org
Website: www.ana-jana.org

Yeast β 1,3-glucan and Its Use Against Anthrax Infection and in the Treatment of Cancer

Russell L. Blaylock, MD,
Member, *JANA* Editorial Board
Clinical Assistant Professor,
University of Mississippi Medical Center, Jackson, Mississippi

In this edition of *JANA*, the paper by Vetvicka *et al.* makes an important contribution to our scientific understanding of the nutraceutical stimulation of the immune system in the treatment of both infectious disease and cancer. While abundant evidence demonstrates the ability of β 1,3-glucans to activate macrophages and neutrophils when given intravenously or intraperitoneally, there has been little information concerning its efficiency when given orally.

In their study, Vetvicka *et al.* used oral β 1,3-glucan (Imucell™ WGP Beta Glucan) from a yeast source in mice infected with *Bacillus anthracis*. With the high incidence of complications associated with anthrax vaccines, an alternative approach is badly needed in this era of bioterrorism threat. Dr. Ken Alibek, a top-ranking scientist at the Russian bioweapons labs, stated that because of the number of possible bioweapon agents available, something other than mass inoculations would be needed. He suggested non-specific immune stimulation. The most effective form of non-specific immune stimulation is macrophage activation.

The anthrax bacillus secretes two toxins, edema toxin and lethal toxin. Edema toxin stimulates an outpouring of fluid, especially into the lungs. Lethal toxin, inhibits neutrophil phagocytosis and triggers destructive intracellular reactions that destroy macrophage cells. Of primary interest is the fact that anthrax lethal toxin inhibits the macrophages from releasing their immune messengers, primarily IL-1, IL-2, IFN-gamma, and TNF-alpha.

Of particular importance in combating infection is the cytokine TNF-alpha. Vetvicka *et al.* demonstrated that yeast-derived β 1,3-glucan given orally stimulates TNF-alpha

release from the macrophage, apparently overcoming inhibition by anthrax lethal toxin. This would account for the high survival figures in the β 1,3-glucan-treated animals. Some previous studies found no increase in TNF-alpha but a significant increase in IL-1 β .¹ Other researchers have demonstrated increased TNF-alpha in response to β -glucan stimulation.²

My own review of the literature confirms their statement that the most effective source of β 1,3-glucan is from *Saccharomyces cerevisiae*, the one chosen by most researchers. Purity of the product is vital, since protein contaminants, as seen in the earlier-used source Zymosan, can cause untoward immune reactions.

β 1,3-glucan also stimulates phagocytosis of neutrophils. In one study, the killing efficiency of neutrophils was increased 20- to 50-fold.³ This is important since the capsular antigen poly-D-glutamic acid from the anthrax organism inhibits neutrophil phagocytosis. It is the two lethal toxins and the capsular antigen that makes the anthrax organism especially deadly. In addition, β 1,3-glucan has been shown to increase clearance of bacteria by the reticuloendothelial system. Thus far, no other solutions have solved this problem.

As for β 1,3-glucan's effects on tumor growth, several studies have shown a significant effect on tumor growth in animal models.^{4,5} Early studies using immune stimulation found occasional tumor growth enhancement. This was later found to be secondary to stimulation of blocking antibody production. A safer and more effective method of immune stimulation is directed at cellular immunity, in particular the stimulation of T-helper cells and NK cells.

β 1,3-glucan has been shown to increase lymphocyte production, NK cell activation, and activation of macrophages. Several studies have also demonstrated the role played by cytokines in inhibiting tumor growth; again, particular interest is in TNF-alpha release.⁶ Of interest also is the role played by IL-1 β , which is increased by β 1,3-glucan as well. Interleukin 1 β has been shown to enhance mobilization of PMLs in the bone marrow and enhance their chemotactic ability. In addition, IL-1 β increases the lymphocyte count and increases their activity.⁷

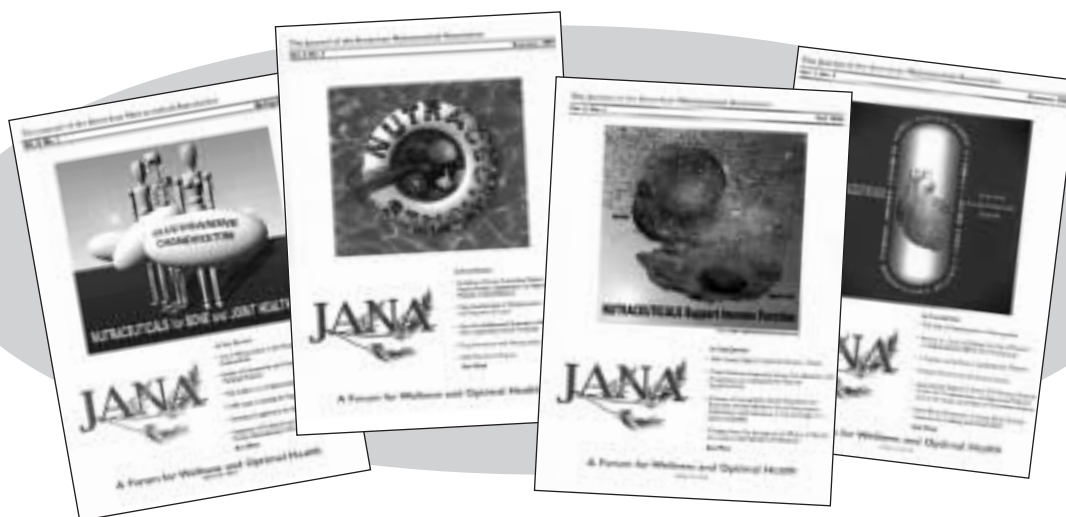
The use of β 1,3-glucan is of special interest in the cancer patient undergoing chemotherapy and/or radiation treatment, since β -glucans have shown a remarkable ability to accelerate hematopoietic recovery in both sublethally and lethally irradiated mice, even when given after the radiation dose. It can also stimulate recovery of the bone marrow following chemotherapy, something vital to restricting tumor growth and preventing infectious complications during treatment.

While data provided in the research by Vetvicka and co-workers is preliminary and needs to be confirmed by a larger controlled trial, this is an important pilot study, in that it demonstrates the effectiveness of oral β 1,3-glucan in treating both infectious agents and tumors.

REFERENCES

1. Rasmussen L-T, Seljelid R. Novel immunomodulators with pronounced *in vivo* effects caused by stimulation of cytokine release. *J Cellular Biochem.* 1991;46:60-68.
2. Sherwood ER, Williams DL, Di Luzio NR. Glucan stimulates production of antitumor cytolytic/cytostatic factors(s) by macrophages. *J Biol Response Modifiers.* 1986;5:504-526.
3. Onderdonk AB, Cisneros RL. *et al.* Anti-infective effect of poly- β -1,6-glucotriosyl- β 1,3-glucopyranose glucan *in vivo.* *Infection and Immunity.* 1992;60:1642-1647.
4. Mansell PWA, Ichinose H, *et al.* Macrophage-mediated destruction of human malignant cells *in vivo.* *J Nat Cancer Inst.* 1975;54:571-576.
5. Sherwood ER, Williams DL, Di Luzio NR. Glucan stimulates production of antitumor cytolytic/cytostatic factors(s) by macrophages. *J Biol Response Modifiers.* 1986;5:504-526.
6. Carswell EA, Old LJ, *et al.* An endotoxin-induced serum factor that causes necrosis of tumors. *Proc Natl Acad Sc.* 1975;72:3666-3670.
7. Browder W, Williams D, *et al.* Beneficial effect of enhanced macrophage function in the trauma patient. *Ann Surg.* 1990;211:605-613.

SUBSCRIBE TODAY TO THE LEADING JOURNAL ON NUTRACEUTICAL SCIENCE



The Journal of the American Nutraceutical Association (JANA)

To subscribe to JANA - Phone 800-566-3622
(outside the USA, 205-833-1750),
or visit the ANA website at www.ana-jana.org

Pilot Study: Orally-Administered Yeast β 1,3-glucan Prophylactically Protects Against Anthrax Infection and Cancer in Mice

Vaclav Vetvicka, PhD,¹ Kiyomi Terayama, MD,² Rosemonde Mandeville, MD, PhD,³
Pauline Brousseau, PhD,³ Bill Kournikakis, PhD,⁴ Gary Ostroff, PhD^{5*}

¹Department of Pathology, School of Medicine, University of Louisville, Louisville, Kentucky;

²Department of Pathology, Tokyo Dental College,

Ichikawa General Sugano Ichikawa City, Chiba Prefecture, Japan;

³Biophage Pharma Inc., Montreal, Quebec Canada; ⁴Defence Research Establishment Suffield, Ralston, Alberta Canada; ⁵Biopolymer Engineering, Inc., Eagan, Minnesota

ABSTRACT

β 1,3-glucans from various bacterial, mushroom, yeast, and cereal sources have been established as immunomodulators. In the present paper we demonstrate that orally-administered yeast β 1,3-glucan (WGP Beta Glucan) had significant effects as a prophylactic treatment to reduce the mortality of anthrax infection in mice. In addition, the same type of treatment also inhibited the growth of cancer cells *in vivo*. The mechanism of action involves the stimulation of three important cytokines: IL-2, IFN- γ , and TNF- α . These results provide preclinical evidence for the beneficial effects of orally-administered yeast β 1,3-glucan.

INTRODUCTION

β 1,3-glucan's role as a biologically active immunomodulator has been well documented for over 40 years. First interest in the immunomodulatory properties of polysaccharides was raised after experiments showing that a crude yeast cell preparation stimulated macrophages via activation of the com-

plement system.¹ Further work identified the immunomodulatory active component as β 1,3-glucan.² Numerous studies have subsequently shown that β 1,3-glucans, either particulate or soluble, exhibit immunostimulating properties, including antibacterial and anti-tumor activities.^{3,4}

β 1,3-glucans can be isolated from almost every species of yeast. β 1,3-glucan derived from *Saccharomyces cerevisiae* (Baker's yeast) has been the most extensively studied. β 1,3-glucan forms a significant part of the yeast cell wall, together with mannan, proteins, lipids, and small amounts of chitin. In addition to yeast, β 1,3-glucans can be isolated from bacteria, mushrooms, algae, or cereal grains. The structure of the β 1,3-glucan depends on both source and type of isolation. Different physicochemical parameters, such as solubility, primary structure, molecular weight, and branching play a role in the biological activities of β 1,3-glucans.⁵

Original studies on the effects of β 1,3-glucan on the immune system focused on mice. Subsequent studies demonstrated that β 1,3-glucan has strong immunostimulating activity in a wide variety of other species, including earthworms, shrimps, fish, chicken, rats, rabbits, guinea pigs, sheep, pigs, cattle, and humans (for review see reference 6). Based on these results it has been concluded that β 1,3-glucan represents a type of immunostimulant that is active across the evolutionary spectrum, likely representing an evolutionarily-conserved innate immune response directed against fungal pathogens.⁷⁻⁹

* Correspondence:

Gary Ostroff

Biopolymer Engineering, Inc.

3388 Mike Collins Drive

Eagan, MN 55121

Phone: 651-675-0300 Fax: 651-675-0400

Email: gostroff@biopolymer.com

More than 800 publications have reported that β 1,3-glucans, either soluble or particulate, exhibit immunomodulatory properties. Despite the extensive investigations, no consensus on the source, size, and other properties of β 1,3-glucan has been achieved. In addition, numerous concentrations and routes of administration have been tested, including intraperitoneal, subcutaneous, and intravenous applications. For decades, oral treatment with β 1,3-glucan has been on the periphery of interest, despite the fact that it represents the most convenient route. In the last decade, however, a renewed interest in human application brought about important studies of orally-administered β 1,3-glucan.

In this paper we report on the activity of orally-delivered yeast β 1,3-glucan (WGP Beta Glucan) in infectious disease and tumor animal model systems. Although the anti-infective properties of β 1,3-glucans have been already established, the majority of this work has been done with parenterally-administered β 1,3-glucans, and the number of bacteria tested were very limited. Due to the recent threats of bioterrorism, we tested the effects of orally-delivered yeast β 1,3-glucan on infection with *Bacillus anthracis*. Similarly, the anti-tumor effects of β 1,3-glucans are well established, and most of the work has been done with parenterally-administered fungal β 1,3-glucans, or oral administration of crude mushroom glucan preparations. In this study we tested the effects of oral administration of highly purified yeast β 1,3-glucans on tumor growth.

MATERIAL AND METHODS

Yeast β 1,3-glucan

A highly purified particulate yeast-derived β 1,6-branched β 1,3-glucan (Imucell™ WGP Beta Glucan, Biopolymer Engineering, Inc., Eagan, Minn.) was used in all experiments. The glucan was suspended in water at indicated concentrations.

Bacteria

Bacillus anthracis strain Vollum 1B (USAMRIID, Ft. Detrick, Md) was used for preparation of spores by harvesting from blood agar plates followed by heat shock (80°C for 11 minutes). Aliquots diluted in phosphate buffered saline (PBS) were stored at -80°C. A well-established model of anthrax infection in mice was used.¹⁰

Mice

Female, 6-wk-old BALB/c mice were purchased from Charles River Laboratories, St. Constant, Quebec, or male 6-wk-old BALB/c mice were purchased from Clea Japan, Inc. For anthrax experiments, mice were maintained inside the biosafety level 3 laboratory at DRES. Care and handling of mice followed guidelines set out by the Canadian Council on Animal Care.

Tumor cell line

Mouse intestinal tumor cell line Colon26 was passaged

in vivo in BALB/c mice.¹¹ Twenty-one days after inoculation, the tumors were excised, gently teased over stainless steel screens, washed in RPMI 1640 medium and resuspended in PBS (1x10⁶ viable cells/ml).

Anthrax-protective prophylactic effects

Experimental groups (10 mice/group) were gavaged daily (days -7 to 0) with 0.1 ml of water containing either 0, 2 mg/kg or 20 mg/kg of WGP Beta Glucan. On day 0, 60 minutes after the final oral treatment, mice were infected subcutaneously with an LD₆₀ dose of 85 ± 11 anthrax spores/animal. Confirmation of the doses was determined by seeding 0.1 ml of the same suspension on blood agar plates. All experimental animals were monitored twice daily post-infection for 10 days. At day 10, mortality had plateaued and the experiment was ended.

Tumor-protective effects

Tumor cells (1x10⁵ viable cells in 0.1 ml) were injected into the abdominal wall of all animals on day 0. Groups of mice (8 mice/group) were gavaged daily with water or WGP Beta Glucan (28.4 mg/kg) for 21 consecutive days (days 1-21). Twenty-one days after tumor administration animals were sacrificed, tumors excised, and tumor weight measured.

Chemicals

Fetal calf serum and RPMI 1640 medium were purchased from Gibco BRL (Rockville, MD), HEPES, Concanavalin A, and lipopolysaccharide were purchased from Sigma (St. Louis, MO).

Cytokines

For evaluation of cytokine production, we incubated purified spleen cells from each animal (2x10⁶ cells/ml in RPMI 1640 medium with 5% FCS) in wells of a 24-well tissue culture plate. After addition of 1 µg of Concanavalin A or 10 µg of LPS per well, cells were incubated for 72 hr in a humidified incubator (37°C, 5% CO₂). At the endpoint of incubation, supernatants were collected, filtered through 0.45 µm filter and tested for the presence of IL-2, IFN-γ and TNF-α. Levels of cytokines were measured by commercial ELISA in accordance with the protocol for Cytoscreen™ of BioSource International, Inc. (Camarillo, CA).

RESULTS

Orally-administered WGP Beta Glucan treatments showed significant anthrax-protective anti-infective effects. Under our experimental conditions, 5 out of 10 control mice survived the anthrax infection. Compared to this 50% control survival rate, prophylactic daily oral doses of WGP Beta Glucan (2 or 20 mg/kg) increased survival to 100% (Figure 1).

Orally-administered WGP Beta Glucan treatments also showed tumor-protective effects on tumor size and vascularization (Figure 2). Table 1 shows the effects of oral administration of WGP Beta Glucan on tumor weight. These

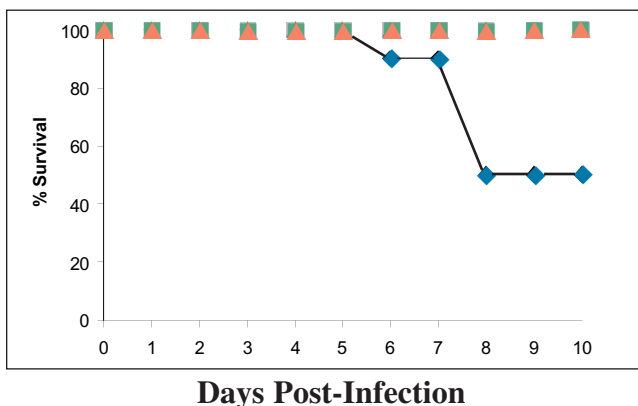
results show the beneficial effects of WGP Beta Glucan, as evidenced by a statistically significant decrease (-21%) in tumor weight at day 21 from 0.66 +/- 0.06 g in control mice to 0.52 +/- 0.06 g in β 1,3-glucan-treated mice ($P < 0.05$). In addition, the control animals were observed to be inactive and crouching, and had reduced body temperature in comparison to the WGP Beta Glucan-treated animals.

Evidence of orally-administered WGP Beta Glucan immunomodulatory activity was also demonstrated through effects on the production of three different cytokines, IL-2, IFN- γ , and TNF- α (Table 2). The production of these three cytokines was measured after a 72 hr *in vitro* incubation of spleen cells isolated from control and WGP Beta Glucan-administered animals. For all three tested cytokines, oral administration of WGP Beta Glucan resulted in significantly-increased cytokine levels (IL-2 (2.3-fold), IFN- γ (4.4-fold), and TNF- α (2.2-fold), $P < 0.05$) over control animals.

DISCUSSION

β 1,3-glucan is widely used as a dietary supplement, with well-established stimulating effects on the immune defense system.^{6,12} A large body of published data supports this use. Browder *et al.* described strongly decreased septic morbidity with β 1,3-glucan administration.¹³ A series of well-documented multicenter blind studies demonstrated that β 1,3-glucan-treated patients had significantly lower infection rates.^{14,15} Positive effects were also found in patients after cardiopulmonary bypass,¹⁶ and inhibition of

Figure 1. Anthrax-protective effect of daily oral prophylactic administration of WGP Beta Glucan.



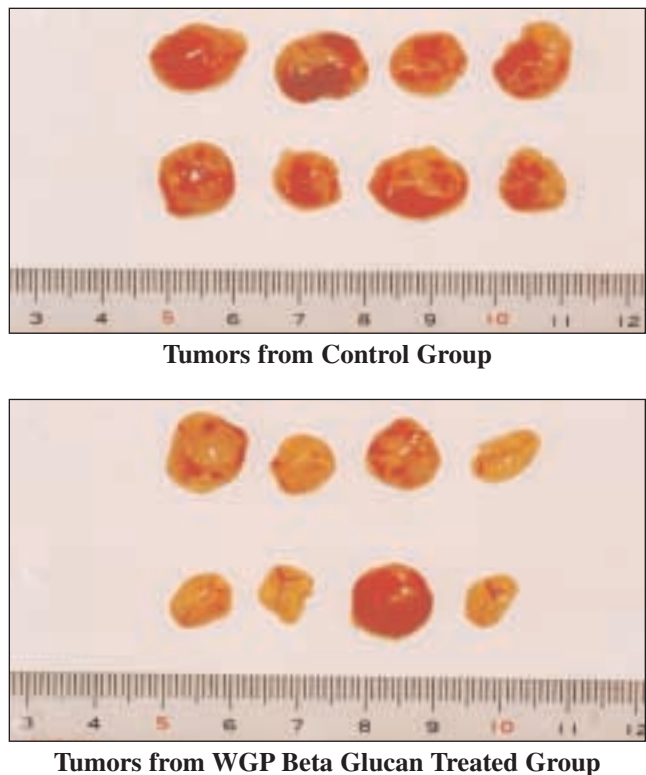
Groups of 10 Balb/c mice were gavaged daily (days -7 to 0) with 0.1 ml of water as a control (◆) or 0.1 ml water containing 40 (■) or 400 µg (▲) of WGP Beta Glucan per mouse (2 or 20 mg/kg). On Day 0, one hour after the last prophylactic oral dosing, animals were infected subcutaneously with an LD₆₀ dose of *B. anthracis* spores. Animals were observed daily until the end of the study and survival time recorded. The percentage survival was calculated from the ratio of surviving animals each day to the total number of challenged animals in each group (n = 10). *P values were determined using a Fisher exact test (daily prophylactic 2 and 20 mg/kg; $P = 0.016$).

antiviral activity has been found in HIV-infected patients.¹⁷ Some β 1,3-glucans are routinely used in patients for tumor immunotherapy.^{18,19}

The majority of the previous work has dealt with injected β 1,3-glucans. Only a limited number of investigations have focused on oral administration. Suzuki *et al.* demonstrated significant activation of peritoneal macrophages by orally-delivered β 1,3-glucan.^{20,21} In a subsequent communication they reported an enhancement of alveolar macrophage function by oral delivery of β 1,3-glucan,²² probably mediated via the Peyer's patches in the intestinal wall. Oral administration of lentinan has been found to increase the number of T helper cells in blood of lentinan-fed rats.²³ To further study the mechanism of action of orally-administered β 1,3-glucans, Ikuzawa *et al.* studied the fate and tissue distribution of Krestin.²⁴ Based on these promising reports, latter attention focused on effects of β -glucans delivered *per os*. The summation of this work suggests that β 1,3-glucans function by stimulating host immune defense mechanisms, primarily macrophages, neutrophils, and NK cells.

With the threat of bioterrorism in the United States

Figure 2. Tumor-protective effect of daily oral administration of WGP Beta Glucan.



Tumor cells were implanted into all animals by abdominal wall injection on Day 0. Groups of mice (8 mice/group) were gavaged daily with water or WGP Beta Glucan (28.4 mg/kg) for 21 consecutive days (days 1 to 21). Twenty-one days after tumor administration animals were sacrificed, tumors excised and photographed.

Table 1. Effect of oral administration of WGP Beta Glucan on tumor growth

	Control	WGP Beta Glucan
Tumor Weight (g)	0.66 +/- 0.06	0.52 +/- 0.06*

At day 21 after tumor cell administration animals were sacrificed, tumors excised, and weighed. *P values were determined using a student's T-test ($p < 0.05$).

becoming a reality, we tested the hypothesis that oral yeast β 1,3-glucan could be used as a protective agent against anthrax infection. The preclinical results described in this report demonstrate that orally-administered WGP Beta Glucan has strong anthrax-protective effects. Oral WGP Beta Glucan treatment significantly increased the number of surviving animals as well as prolonged survival time of lethally-infected animals. Dose ranging studies to date have demonstrated that daily prophylactic doses of 2-20 mg/kg WGP Beta Glucan provides a maximal anthrax-protective effect in mice. Ongoing dose ranging studies are being conducted to determine the minimal WGP Beta Glucan dose required to protect mice against a lethal anthrax infection. Based on the presented preclinical data, WGP Beta Glucan shows promise as a prophylactic treatment to support the immune system and reduce the risk of anthrax infection.

Oral WGP Beta Glucan treatment also reduces the threat of cancer, slowing down the progression of tumor growth in a preclinical colon cancer model. This observation extends the large body of preclinical and clinical work done in Japan demonstrating the oral anti-tumor activity of mushroom β 1,3-glucans²⁵⁻²⁷ to yeast β 1,3-glucan. These published studies have demonstrated that β 1,3-glucan immunotherapy leads to the activation of the innate immune cells (macrophages, neutrophils (PMN) and natural killer (NK) cells), the stimulation of tumoricidal activities, production of cytokines, and the generation of enhanced cell-mediated responses. Suzuki and colleagues have reported the stimulation of activated macrophages by the administration of SSG²² and NK-type lymphokine-activated killer cells by the combined administration of lentinan and IL-2.²⁸ The stimulation of tumoricidal activities in PMN by a linear bacterial β 1,3-glucan has also been reported.^{29,30} A number of clinical studies have demonstrated synergy between oral β 1,3-glucan immunotherapy, and traditional radiation and chemotherapeutic cancer treatment options.^{31, 32}

At present we do not fully understand the mechanisms mediating the anthrax and tumor-protective effects of WGP Beta Glucan. We believe that through specific interactions between the β 1,3-glucan active component of WGP Beta Glucan and β 1,3-glucan receptors on M-cells within Peyer's patches in the intestinal mucosa that a systemic signal provided by cytokines is elicited by the gut-associated lymphatic system that stimulates the innate immune system

Table 2. Effects of oral administration WGP Beta Glucan on cytokines

Cytokine	Control	WGP Beta Glucan
IL-2	9.7 +/- 0.5 pg/ml	23.4 +/- 2.1 pg/ml*
IFN-γ	107.8 +/- 8.4 pg/ml	475.8 +/- 42.3 pg/ml*
TNF-α	487.8 +/- 58.2 pg/ml	1083.5 +/- 44.6 pg/ml*

Twenty-one days after tumor cell administration, animals were sacrificed, spleens excised, and spleen cells purified from each animal. Spleen cells from each animal were cultured with 1 μ g of Concanavalin A or 10 μ g of LPS for 72hr. Culture supernatants were collected, filtered through 0.45 μ m filter, and tested for presence of IL-2, IFN- γ , and TNF- α . *P values were determined using a student's T-test ($P < 0.05$ for IL-2, IFN- γ , and TNF- α).

components (macrophages, neutrophils, and NK cells) to a higher functional level, increasing the first line of host defense mechanisms. For these experiments we focused on three important cytokines, IL-2, IFN- γ , and TNF- α . All of these cytokines play an important role not only in physiological processes, but also in bioregulation of host defense reactions. IL-2 is a cytokine produced by activated CD4 and some CD8 T lymphocytes. In addition to being the major T cell growth factor, IL-2 also stimulates: growth and differentiation of cytotoxic T cell precursors, NK cells, differentiation of activated human B-lymphocytes, and activation of monocytes. TNF- α is a pleiotropic cytokine secreted primarily by monocytes/macrophages and T lymphocytes, respectively. TNF- α is the principal mediator of natural immunity against gram-negative bacteria and a key mediator of inflammatory responses and septic shock.³³ IFN- γ , sometimes called immune interferon, is produced mainly by T lymphocytes as a result of antigenic or mitogenic stimulation. The activities of IFN- γ are many, including induction of MHC expression, macrophage activation, and effects on the differentiation of lymphocytes.

In this paper we report important biological activities of yeast-derived β 1,3-glucan (ImucellTM WGP Beta Glucan). Oral administration of WGP Beta Glucan increased the production of three important cytokines (IL-2, IFN- γ , and TNF- α), inhibited growth of cancer cells *in vivo*, and provided a prophylactic defense against anthrax infection in mouse models. Despite our current lack of knowledge about the precise mechanisms through which oral β 1,3-glucan mediates its protective effects, these anti-tumor and anti-infective properties of yeast-derived WGP Beta Glucan presented in this report suggest that further study is warranted to understand these benefits of β 1,3-glucans.

REFERENCES

1. Benacerraf B, Sebestyen MM. Effect of bacterial endotoxins on the reticuloendothelial system. *Fed Proc.* 1957;16:860-867.
2. Rigi SJ, Di Luzio NR. Identification of a reticuloendothelial stimulating agent in zymosan. *Am J Physiol.* 1961;200:297-300.

3. Di Luzio NR, Williams DL, McNamee RB, Edwards BF, Kitahama A. Comparative tumor-inhibitory and anti-bacterial activity of soluble and particulate glucan. *Int J Cancer*. 1979; 24:773-779.
4. Imura H, Ohno N, Suzuki I, Yadomae T. Purification, antitumor activity, and structural characterization of β -1,3-glucan from *Peziza vesiculosa*. *Chem Pharm Bull*. 1985;33:5096-5099.
5. Yadomae T. Structure and biological activities of fungal β -1,3-glucans. *Yakugaku Zasshi*. 2000;120:413-431.
6. Vetvicka V. β -glucans as immunomodulators. *JANA*. 2001;3:24-31.
7. Wilson R, Chen C, Ratcliffe NA. Innate immunity in insects: the role of multiple, endogenous serum lectins in the recognition of foreign invaders in the cockroach, *Blaberus discoidalis*. *J Immunol*. 1999;162(3):1590-1596.
8. Kim YS, Ryu JH, Han SJ, Choi KH, Nam KB, Jang IH, Lemaitre B, Brey PT, Lee WJ. Gram-negative bacteria-binding protein, a pattern recognition receptor for lipopolysaccharide and beta-1,3-glucan that mediates the signaling for the induction of innate immune genes in *Drosophila melanogaster* cells. *J Biol Chem*. 2000;275(42):32721-32727.
9. Takaki Y, Seki N, Kawabata S, Iwanaga S, Muta T. Duplicated binding sites for (1-3)-beta-D-glucan in the horseshoe crab coagulation factor G: Implications for a molecular basis of the pattern recognition in innate immunity. *J Biol Chem*. 2002;277:14281-14287.
10. Welkos SL, Keener TJ, Gibbs PH. Differences in susceptibility of inbred mice to *Bacillus anthracis*. *Infect Immun*. 1986; 51:795-800.
11. Ogasawara M, Murata J, Kamitani Y, Hayashi K, Saiki I. Inhibition by vasoactive intestinal polypeptide (VIP) of angiogenesis induced by murine Colon 26-L5 carcinoma cells metastasized in liver. *Clin Exp Metastasis*. 1999;17:283-291.
12. Ross GD, Vetvicka V, Yan J, Xia Y, Vetvicková J. Therapeutic intervention with complement and β -glucan in cancer. *Immunopharmacol*. 1999;42:61-74.
13. Browder W, Williams D, Pretus H, Olivero G, Enrichens F, Mao P, Franchello A. Beneficial effect of enhanced macrophage function in the trauma patient. *Ann Surg*. 1990; 211:605-613.
14. Babineau TJ, Hackford A, Kenler A, Bistran B, Forse RA, Fairchild PG, Heard S, Kerorack M, Causha P, Benotti P. A phase II multicenter, double-blind, randomized, placebo-controlled study of three dosages of an immunomodulator (PGG-glucan) in high-risk surgical patients. *Arch Surg*. 1994;129: 1204-1210.
15. Babineau TJ, Marcello P, Swails W, Kenler A, Bistran B, Forse RA. Randomized phase I/II trial of a macrophage-specific immunomodulator (PGG-glucan) in high-risk surgical patients. *Ann Surg*. 1994;220:601-609.
16. Hamano K, Gohra H, Katoh T, Fujimura Y, Zempo N, Esato K. The preoperative administration of lentinan ameliorated the impairment of natural killer activity after cardiopulmonary bypass. *Int J Immunopharmacol*. 1999;21:531-540.
17. Itoh W, Sugawara I, Kimura S, Tabata K, Hirata A, Kojima T, Mori S, Shimada K. Immunopharmacological study of sulfated schizophyllan (SPG) I. Its action as a mitogen and anti-HIV agent. *Int J Immunopharmacol*. 1990;12:225-233.
18. Kimura Y, Tojima H, Fukase S, Takeda K. Clinical evaluation of sizofilan as assistant immunotherapy in treatment of head and neck cancer. *Acta Otolaryngol*. 1994;511 (suppl):192-195.
19. Nakano T, Oka K, Hanba K, Morita S. Intratumoral administration of sizofilan activates Langerhan cell and T-cell infiltration in cervical cancer. *Clin Immunol Immunopathol*. 1996; 79:79-86.
20. Suzuki I, Hashimoto K, Ohno N, Tanaka H, Yadomae T. Immunomodulation by orally administered β glucan in mice. *Int J Immunopharmac*. 1989;11:761-769.
21. Sakurai T, Hashimoto K, Suzuki I, Ohno N, Oikawa S, Masuda A, Yadomae T. Enhancement of murine alveolar macrophage functions by orally administered β -glucan. *Int J Immunopharmac*. 1992;14:821-830.
22. Haneue H, Tokuda Y, Machimura T, Kamijoh A, Kondo Y, Ogoshi K, Makuuchi H, Nakasaki H, Tajima T, Mitomi T, Kurosawa T. Effects of oral lentinan on T-cell subsets in peripheral venous blood. *Clin Therapeut*. 1989;11:614-623.
23. Ikuzawa M, Matsunaga K, Nishiyama S, Nakajima S, Kobayashi Y, Andoh T, Kobayashi A, Ohhara M, Ohmura Y, Wada T, Yoshikumi C. Fate and distribution of an antitumor protein-bound polysaccharide PSK (Krestin). *Int J Immunopharmacol*. 1988;10:415-423.
24. Torisu M, Hayashim Y, Ishimitsu T, Fujimura T, Iwasaki K, Katano M, Yamamoto H, Kimura Y, Takesue M, Kondo M, Nomoto K. Significant prolongation of disease-free period gained by oral polysaccharide K (PSK) administration after curative surgical operation of colorectal cancer. *Canc Immunol Immunother*. 1990;31:261-268.
25. Xu G. The effects of PSP on improving immunity for gastric cancer patients In: Yang Q, Kwok C, eds. *PSP International Symposium*. Hong Kong: Fudan University Press; 1993:263-264. Abstract.
26. Nanba H. Results of non-controlled clinical study for various cancer patients using maitake D-fraction. *Explore*. 1995;6:19-21.
27. Suzuki I, Sakurai T, Hashimoto K, Oikawa S, Masuda A, Ohsawa M, Yadomae T. Inhibition of experimental pulmonary metastasis of Lewis lung carcinoma by orally administered β -glucan in mice. *Chem Pharm Bull*. 1991;39:1606-1608.
28. Suzuki I, Tanaka H, Kinoshita A, Oikawa S, Osawa M, Yadomae T. Effect of orally administered β -glucan on macrophage function in mice. *Int J Pharmacol*. 1990;12: 675-684.
29. Morikawa K, Kamegaya S, Yamazaki M, Mizuno D. Hydrogen peroxide as a tumoricidal mediator of murine polymorphonuclear leukocytes induced by a linear beta-1,3-D-glucan and some other immunomodulators. *Cancer Res*. 1985 45(8):3482-3486.
30. Kasai S, Fujimoto S, Nitta K, Baba H, Kunimoto T. Antitumor activity of polymorphonuclear leukocytes activated by a beta-1,3-D-glucan. *J Pharmacobiodyn*. 1991;14(9):519-25.
31. Mitomi T, Tsuchiya S, Iijima N, Aso K, Suzuki K, Nishiyama K, Amano T, Takahashi T, Murayama N, Oka H, Oya K, Noto T, Ogawa N, Randomized, controlled study on adjuvant immunotherapy with PSK in curatively resected colorectal cancer. *Dis Colon Rectum*. 1992;35:123-130.
32. Hayakawa K, Mitsunashi N, Saito Y, Takahashi M, Kanato S, Shiojima K, Furuta M, Niibe H. Effect of krestin (PSK) as adjuvant treatment of the prognosis after radical radiotherapy in patients with non-small cell lung cancer. *Anticancer Res*. 1993;13:1815-1820.
33. Vassalli P. The pathophysiology of tumor necrosis factors. *Annu Rev Immunol*. 1992;10:441-452.

ACKNOWLEDGEMENTS

We thank Dr. Lucie Richard, Isabelle Lussier, Anne Larrivée, Marie-Soleil Christin, and Annie Venne from Biophage Pharma, Inc.; and Nicole Stady, Laurel Negrych, and Dr. John Cherwonogrodzky from DRES for their excellent work with the anthrax experiments; and Kaoru Hayashi for his excellent work with the tumor and cytokine experiments. This project was supported by DRDC, Defence Industrial Research (DIR), Biophage Pharma, Inc., Biopolymer Engineering, Inc., and Men-Eki Ohyo Kenkyusho.