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β-Glucan ameliorates the effects of several infections

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Abstract

Background: Glucans are known for their effects on anti-infection immunity. However, the individual papers always focused on one glucan, and no attempts were ever made to use one particular glucan in numerous infection.

Methods: In our study, we use yeast-derived glucan #300, previously shown to ameliorate the effects of two different infections, to evaluate its anti-infection effects on mouse model of *Mycobacteria bovis*, *Aeromonas hydrophila* and *Escherichia coli* infection. Effects of supplementation was evaluated after 14 days of oral suppelentation with glucan.

Results: In case of *E. coli* infection, we found glucan treatment achieved strong improvements in lowering numbers of bacterial in the spleen. Similar effects were found in the number of specific antibodies and survival. Similarly, glucan supplementation caused significant reduction of tissue bacterial load in *Aeromonas* infection and the spleen of *Mycobacterium bovis* infected mice.

Conclusions: Our results clearly demonstrated that once the glucan significantly improved anti-infection reaction of mice.

Keywords: Glucan, infection, immunity, survival, supplementation

Introduction

Natural products, useful in treating and/or preventing numerous diseases, have been sought throughout mankind's history. Currently, there are more than 50,000 dietary supplement products marketed just in the United States alone and over 50% of the American population takes at least one dietary supplement each day. Most of these natural products are plagued with a common problem, i.e., the fact that they often represent a complex mixture of individual ingredients each of which can contribute to their biological activity. Natural (1,3)-β-D-glucans (glucan thereafter)isolated from yeast, grain and mushrooms are well-established biological response modifiers [1,2] representing highly conserved structural components of cell walls. Glucan's role as a natural immunomodulator has been well documented for over 50 years. Interest in the immunomodulatory properties of polysaccharides was initially raised after experiments showing that a crude yeast cell preparation stimulated macrophages via activation of the complement system [3].

The effects of glucan on anti-infection immunity are among the oldest effects of glucan studied. It has been demonstrated that glucans protect against infection, with both bacteria and protozoa, in several experimental models and was shown to enhance antibiotic efficacy in infections with antibiotic-resistant bacteria. The protective effect of glucans was evidenced in experimental infection with *Leishmania major* and *Leishmania-donovani, Candida albicans, Toxoplasma gondii*, *Streptococcus suis*, *Plasmodium berghei*, *Staphylococcus aureus*, *Escherichia coli*, *Mesocestoides corti*, *Trypanosoma cruzi* and *Eimeria vermiformis* (for review see [4]). Effects of glucan on parasites are summarized here [5]. Some detailed studies have shown significant synergy of glucan with common antibiotics. Original studies done on guinea pigs demonstrated that simultaneous administration of glucan and antibiotics elevated the ability of animals to resist lethal septic infection by antibiotic-resistant bacteria [6]. In addition to hundreds of reports on glucans stimulating the immune response against many infections, there have been numerous studies, including clinical trials, conducted with glucan and infections in humans. Alpha-Beta Technologies conducted a series of human trials in the 1990s. Using double blind, placebo-controlled trials, these studies showed that patients who received glucan had significantly less infections, a decrease in the use of antibiotics, less complications

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and a shorter stay in the intensive care unit [7]. Subsequent trials including a phase III clinical trial showed that glucan therapy reduced serious postoperative infections by 39% after high-risk non-colorectal surgery. Another study focused on vulvovaginal candidiasis, which is among the most prevalent vaginal diseases. The experiments showed that glucan induced reactive oxygen production in human neutrophils and release of important cytokines. It strongly indicates that glucan can be an efficient immunomodulator that triggers an increase in the resistance to various microorganisms [8].

Material and Methods

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 CO_2 asphyxiation. Nine mice/group were used.

Material

Yeast-derived insoluble Glucan #300 was purchased from Transfer Point (Columbia, SC). The purity is over 85%. Auramin O was purchased from Sigma (St. Louis, MO, USA).

Experimental groups

A control group of nine mice was administered PBS. The glucan-supplemented group received 100 mg/mouse of glucan orally for 14 days prior to the experiment.

Infection

Mice were infected by intragastric application of one dose of *Escherichia coli* (Sigma, St. Louis, USA) at $5x10^6$ CFU/ml. In another set of experiments, mice were injected iv. with-*Mycobacteria bovis* ($2x10^5$/mouse in 0.1 ml of PBS) [9]. The optimal dilution was determined in preliminary experiments. The challenge was performed by ip. injection of 100 ml of bacterial suspension containing $5x10^9$ of bacteria in PBS [10]. *Aeromonas hydrophila* strain (ATCC07966) was maintained and subcultured in liquid peptone broth three times before experiments. Mice were orally fed with bacterial suspension of *A. hydrophila* (0.2 ml containing $2x10^8$ CFU/mouse/week) for 4 weeks. A second group obtained both bacteria and glucan administered same way [11].

Antibodies

A total Ig level was measured by an anti-mouse Ig-Px antibodies and ELISA assay using plates coated overnight with a standardized suspension of heat-inactivated *E. coli* at $2x10^8$ CFU/ml in pH 9.0 carbonate/bicarbonate buffer.

Microscopy

Levels of *M. bovis* bacteria were evaluated by fluorescence microscopy. Bacteria were labeled with auramin O in formaldehyde-fixed spleen homogenates at various intervals after the challenge.

Antibiotics

Mice were treated with streptomycin (Sigma) at 5 g/l in drinking water for 7 days.

Statistics

Student's t-test was used to statistically analyze the data. Data at $p<0.05$ were considered significantly different.

Results

First we focused on effects of glucan supplementation on infection with *E. coli*. Results shown in **Figure 1** showed that glucan treatment achieved similar improvements in lowering numbers of bacterial in the spleen as treatment with antibiotics. When we measured the level of specific antibodies, the glucan supplementation or antibiotic treatment has statistically identical effects (**Figure 2**). The survival curve demostrated that both glucan and antibodies similarly protected the infected animals, with the antibodies being slightly more active at the

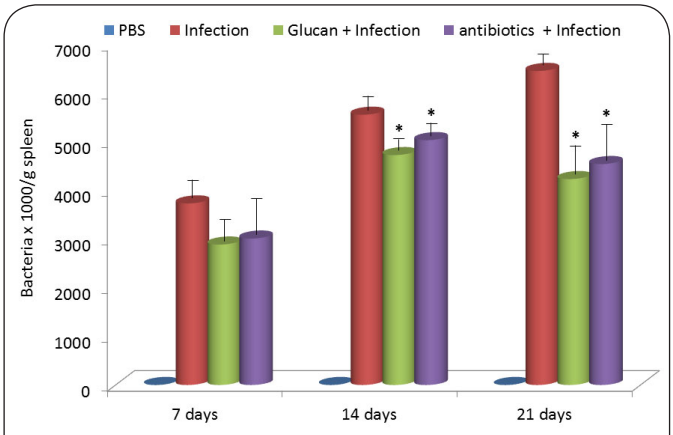

Figure 1. Effect of supplementation with glucan and/or antibiotics on bacteria counts in spleen. Each value represents the mean±SD. *Represents significant differences between control and experimental samples at P≤0.05 level.

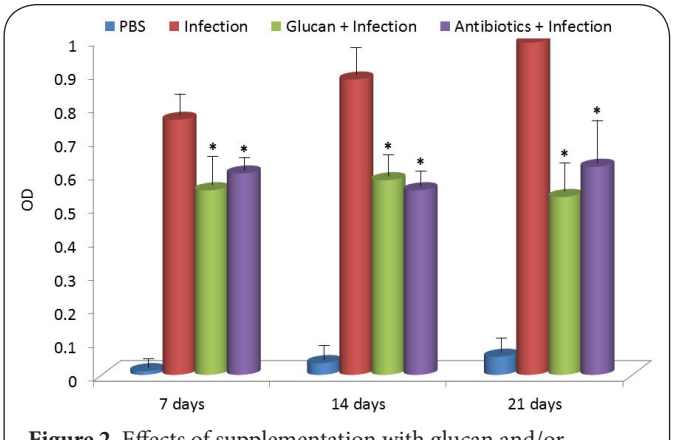

Figure 2. Effects of supplementation with glucan and/or antibiotics on the level of specific antibodies. Each value represents the mean±SD. *Represents significant differences between control and experimental samples at P≤0.05 level.

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end of the study (**Figure 3**).

When we tested the effects on *Aeromonas* infection, glucan was found to strongly reduce the tissue bacterial load (**Figure 4**). As far as survival is concerned, glucan supplementation improved the survival rate from day for till the end of the study (**Figure 5**).

Addition of glucan to the diet also improved the bacterial load in the spleen of *Mycobacterium bovis* infected mice (**Figure 6**), clearly showing the anti-infectious effects of glucan supplementation.

Discussion

Glucans are well-established immunomodulators with marked ability to improve the host response to bacterial, viral or parasitic infections. For details about the protective effects of glucan in parasitic infection seerecent review [5]. Lentinan showed prophylactic effects on experimental tuberculosis

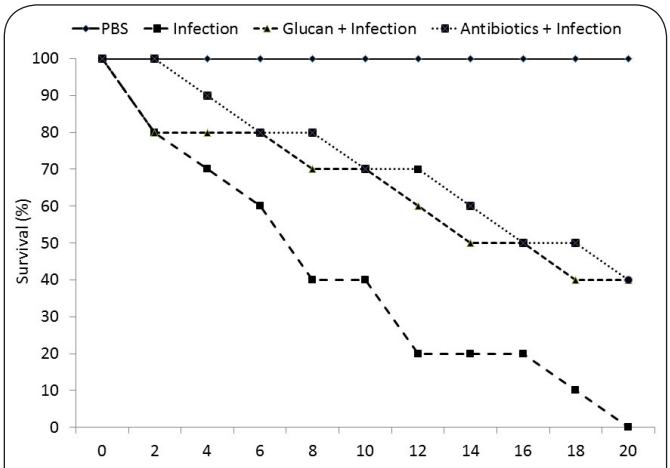

Figure 5. Effects of supplementation with glucan and/or antibiotics on survival rate after *A. hydrophila* infection.

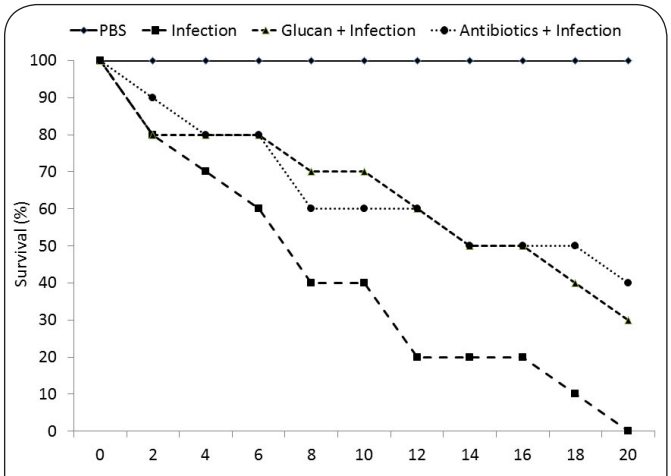

Figure 3. Effects of supplementation with glucan and/or antibiotics on survival rate after *E. coli* infection.

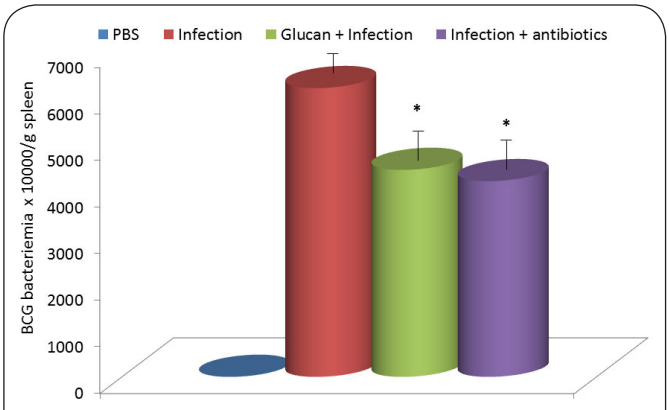

Figure 6. Effects of supplementation with glucan and/or antibiotics on tissue bacterial load in *M. bovis* infection. Each value represents the mean±SD. *Represents significant differences between control and experimental samples at P≤0.05 level.

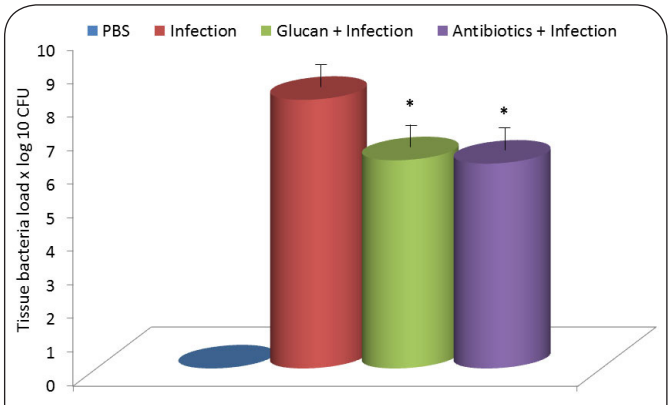

Figure 4. Effects of supplementation with glucan and/or antibiotics on tissue bacterial load in *A. hydrophila* infection. Each value represents the mean±SD. *Represents significant differences between control and experimental samples at P≤0.05 level.

via improvements in defense reactions [12]. Oat glucan administered either parenterally or intragastrically on mice infected with *Eimeria vermiformis* increased formation of specific antibodies and reduced fecal oocyst shedding [13], improvement in case *E. coli* infection were found to be mediated via increase release of numerous cytokines including IL-1 [14]. Purified mushroomglucan ameliorates pulmonary sepsis caused by antibiotic-resistant *Klebsiella* [15], barley glucan showed antibacterial effects in *Staphylococcus* infection [16].

Yeast-derived glucan stimulation was found not only improve the resistance against bacterial infection, but also work in synergy with antibiotics [17]. Browder et al. studied the stimulation of human macrophages in trauma patients and found that glucan therapy strongly decreased septic morbidity. A multicenter, double blind study found the optimal dosage of yeast glucan in high-risk surgical patients [17]. In addition, these studies demonstrated the safety and efficacy

of glucan in surgical patients who underwent major thoracic or abdominal surgery. Since no adverse drug experiences associated with glucan infusion have been found, glucan-treated patients had significantly lower levels of infections. The biological effects of glucan on anti-infectious immunity are two-fold: macrophages are activated to produce various substances (such as H_2O_2), directly killing the bacteria and stimulation of B lymphocytes to produce more antibodies. Original studies done on guinea pigs demonstrated that simultaneous administration of yeast glucan and antibiotics elevated the ability of animals to resist lethal septic infection by antibiotic-resistant bacteria [6]. At the same time, those results suggested that the use of glucan can help to lower the doses of antibiotics in commercial farming, which is particularly important since there is a strong effort to completely abandon the use of antibiotics in all farmed animals. Positive effectsof lentinan were also found in patients after cardiopulmonary bypass [18]. Using double blind, placebo-controlled trials, additional studies showed thatyeast glucan-supplemented patients had significantly less infections, a decrease in the use of antibiotics, less complications and a shorter stay in the intensive care unit [7]. A different approach was used by Svidzinski group. They focused on vulvovaginal candidiasis, which is among the most prevalent vaginal diseases. The experiments showed that algae-derived glucan induced reactive oxygen production in human neutrophils and release of important cytokines. It strongly indicates that glucan can be an efficient immunomodulator that triggers an increase in the microbicidal response in these health problems [8].

Original studies done on guinea pigs demonstrated that simultaneous administration of yeast glucan and antibiotics elevated the ability of animals to resist lethal septic infection by antibiotic-resistant bacteria [6]. At the same time, those results suggested that the use of glucan can help to lower the doses of antibiotics in commercial farming, which is particularly important since there is a strong effort to completely abandon the use of antibiotics in all farmed animals.

In the current study, we decided to evaluate the antibacterial effects of glucan #300. This yeast-derived, insoluble glucan has been previously found to suppress cancer development [19], improved stress [20] and fatigue [21], but antibacterial [22] and antiviral effects [23] were tested only rarely.

Using three different models of infection, we evaluated the effects of glucan supplementation. In all three models we found significant improvements in both bacterial counts and survival rates, which is in agreement with the current literature. It is clear that glucan supports the immune system in all types of infection, which suggest the possibility that glucan will be developed for a possible clinical use in treatment of various infections. In our preliminary experiments we already evaluated the effects of this particular glucan on two different models of infection. Using a model of urinary tract infection, we found significant improvements of the amounts of *E. coli* in the bladder after yeast glucan supplementation [22]. In a model of mouse pneumonia, we found that yeast glucan supplementation lead to significant depression of bacterial counts in BALF [22]. Together with the current study, we conclude that yeast derived, insoluble glucan #300 has broad action against numerous bacterial infections. The exact mechanisms of the anti-infectious action of glucan are currently under investigation.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Authors' contributions	VV	JV
Research concept and design	✓	✓
Collection and/or assembly of data	✓	✓
Data analysis and interpretation	✓	✓
Writing the article	✓	--
Critical revision of the article	✓	✓
Final approval of article	✓	✓
Statistical analysis	--	✓

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