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# Glucan Supplementation Ameliorates Some Health Problems Related to the Development of Lyme disease

**Vaclav Vetvicka, Jana Vetvickova**

University of Louisville, Department of Pathology, 511 South Floyd Street, Louisville, KY 40202, USA.



## Abstract

*Background:* Lyme disease is a multiphasic systemic disorder caused by pathogenic infection with Borrelia burgdorferi. Despite intensive research, the adequate treatment of this disease is problematic.

*Material and Methods:* In our study, we focused on testing the hypothesis that oral supplementation with glucan might ameliorate the Borrelia infection. We measured the ankle swelling, cytokine production, cell infiltration and level of specific antibodies.

*Conclusions:* Glucan, one of the most studied natural immunomodulators, was found to contribute: 1) reducing bacterial load, 2) suppressing arthritis severity, 3) stimulation of immune reactions, particularly Th2-related cytokines.

*Keywords:* Immunity; arthritis; cytokines; glucan

## Introduction

Borelia burgdorferi is the spirochete causing Lyme borreliosis. This disease is a multi system infection disease. One of the symptoms is Lyme arthritis, reported in 30% of the patients [1].

Upon entering the body, it effectively activates both alternative and classical complement pathway without requiring antibodies [2] and stimulates oxidative burst [3]. Recent studies revealed the important role of follistatin-like protein 1 in response to infection, particularly in cytokine production [4]. Disease-susceptible C3H mice represent a good model for experimental B. burgdorferi infection. Presence of IL-17 was demonstrated in human patients with Lyme disease, but animal studies found only limited roles of IL-17 or IL-17 signaling the development of disease [5].

Address for correspondence and reprint requests to:
Vaclav Vetvicka, PhD,University of Louisville,Department of Pathology,511 South Floyd Street,Louisville, KY 40202, USA
Tel:502-852-1612 FAX:502-852-1177
Email: vaclav.vetvicka@louisville.edu



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Borreliae opsonized with complement have been shown to adhere to CR3 receptors [6], which is essential for the induction of calcium mobilization, phagocytosis and oxidative burst induction. As CR3 is one of the main receptors for glucan, we evaluated the possible effects of glucan supplementation on B. burgdorferi infection. Toll-like receptors seem to be involved in Lyme disease progression, which might be mediated via TRIF action on controlling release of both inflammatory and anti-inflammatory cytokines [7].

β-1,3-Glucans are structurally complex homopolymers of glucose, usually isolated from yeast and mushrooms. The isolation from various types of mushrooms was a logical follow-up of the folk remedy use of mushrooms in numerous nations. The number of individual glucans is almost as great as the number of sources used for isolation. There are a number of different physicochemical parameters: 1) solubility, 2) primary structure, 3) molecular weight, 4) branching, 5) polymer charge, 6) influence the

biological activities of β-glucans.No clear-cut relationships were ever established.

Research with β-glucans has shown that they function mostly via stimulation of granulocytes, monocytes, macrophages, and natural killer cells. Two membrane β-glucan receptors that trigger responses to β-glucans have been characterized on a molecular level. The first to be reported was CR3 [8], the second was Dectin-1 receptor [9]. As biological effects of glucans appear to be multifactorial, it is not surprising that glucans also influence the production and secretion of cytokines and antibodies. Even a short supplementation with glucan can significantly improve salivary immunity [10].

The original studies on the effects glucan has on the immune system focused on mice. Subsequent studies demonstrated that glucan has a strong immunostimulating activity in a wide variety of other species, including earthworms, bees, shrimp, fish, chicken, rats, rabbits, guinea pigs, sheep, pigs, cattle and humans. Based on these results, it was concluded that glucans represent a type of immunostimulant that is active over the broadest spectrum of biological species.It is one of the first immunostimulants active across the evolutionary spectrum.

## Methods

### *Animals*

Female, 8 week old C3H/HeJ 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. Ten mice were used in each experiment.

### *Material*

Yeast-derived insoluble Glucan #300 was purchased from Transfer Point (Columbia, SC). The purity is over 85%. Fetal calf serum (FCS) was purchased from Hyclone Laboratories (Logan, UT).

### *Bacteria and infection*

Frozen stock of a virulent isolate of B. burgdorferi strain N40 was used in all experiments. Stocks were added to 7 ml of C-BSKH medium containing 6% rabbit serum and grown to log phase at 32 °C. Spirochete dilutions were based on counting under dark-field microscopy and mice were inoculated in each hindfoodpad with 50 ul of BSKH medium containing 5x104 B.burgdorferi organisms [5].Ankle swelling was measured weekly throughout the infection at the thickest craniocaudal portion of the joint using a metric caliper.

### *Pathology*

Infected mice were sacrificed on days 21 and 42 post infection. One ankle joint and one half of the heart were obtained, fixed and paraffin embedded. Sections were stained with hematoxylin and eosin. Sections wereevaluated for disease severity as described previously [11] on a scale from 0 to 4; 0 representing no inflammation and 4 representing serious inflammation.

### *Cellular infiltration*

Cellular infiltration was measured as described [5]. Briefly, removed ankles with removed excess muscle tissue were placed in 15-ml conical tubes containing 5 ml of PBS, 4% FCS, 75μl (0,03 mg

**Table 1: Ankle and heart severity scores**

| Group | Arthritic score | | Carditis score | |
|---|---|---|---|---|
| | Severity | Type | Severity | Type |
| CONTROL | 3.0± 0.0 | 3.0 ± 0.0 | 4.0 ± 0.0 | 4.0 ± 0.0 |
| GLUCAN | 2.2 ± 0.4* | 2.1 ± 0.6* | 2.7 ± 0.8* | 3.5 ± 0.5 |

Scores were determined from histological sections of tibiotarsal joints and hearts or control and glucan-treated mice Results represent mean ± SD. *Significant differences between the groups at P <0.05 level.

DNase 1 (Sigma, St. Louis, MO) and 50µl of 100 mg/ml collagenase /dispase (Roche, Indianapolis, IN) and rocked for 60 min at room temperature. After being gently flayed apart by forceps, cells were strained through the 70 um filter and washed with PBS. Cells were then stained with appropriate FITC-labeled antibodies and evaluated by FACS.

### *Antibodies*

Titers of IgM and IgG antibodies in sera of infected mice were determined by ELISA as previously described [12].

### *Cytokine Array*

Mouse cytokines were measured in serum samples obtained 14 days post-infection [12] by using protein microarray services by Allied Biotech (Ijamsville, MD). The sera from infected and control mice were collected, filtered through 0.22 µm filter and stored in -80 °C freezer.

## Results

To determine glucan supplementation, we orally-fed all mice in the glucan group with a daily dose of 100mg glucan for the whole course of infection. First, we measured if supplementation plays a role in ankle swelling; we monitored the swelling throughout the infection time course. We found no significant differences over the 42 day infection period (Figure 1). To better evaluate if there are any differences in development of disease we measured histological sections of ankles and hearts. We evaluated the section in a blind manner, scored for their overall level of inflammation (severity score) and for the ratio of neutrophils to macrophages (type score). Lower scores are interpreted as resolving inflammation, higher scores as acute inflammation. Data summarized in Table 1 show that glucan supplementation significantly lowered both scores in joints and severity score in carditis.

To examine the effects of adding glucan to a cellular infiltration diet, we used flow cytometry to analyze cellular infiltration into the joint tissue. Single cell suspensions were made from joint tissue and gated on live cells. Next, inflammatory cells, macrophages, neutrophils and T

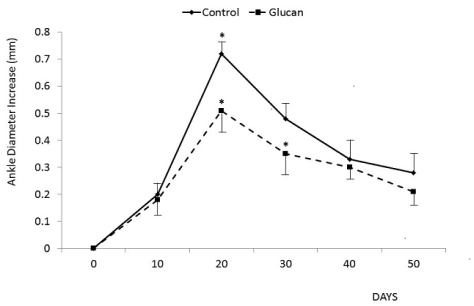


**Figure1: Effect of glucan supplementation on ankle swelling.**

lymphocytes were counted in Day 21 and Day 42. No differences were found on Day 21, but in case of total inflammatory cells, neutrophils and T lymphocytes, significant improvements of infiltrating cells numbers were observed (Figure 2-4). In individual P values for Figure 2 was 0.035, for Figure 3, 0.021 and for Figure 4, 0.023.

ELISA assay against B. burgdorferi whole cell sonicates were performed to determine the antibody profile present in mice after 14 days of infection and glucan supplementation. As shown in Figure 5, there was a significant reduction in total IgM and IgG antibodies following supplementation with glucan.

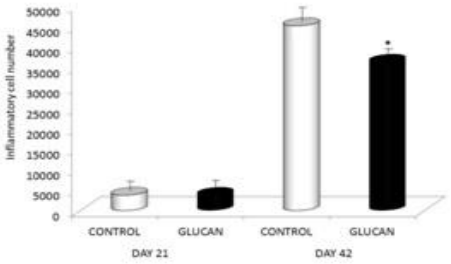


**Figure 2: Cellular infiltrates into infected joints - total inflammatory cells. Data are expressed as mean values ± SD of three independent experiments performed in triplicates. *Represents significant differences between control (PBS) and glucan group at P ≤0.05 level.**

Normal cytokine profile of mice infected with B. burgdorferi is a proinflammatory response with a downregulation of the Th2 response. We performed the cytokine analysis to determine if there was a different cytokine profile in response to glucan supplementation. We found that this treatment significantly upregulated the release of IL-4, IL-5, IL-9, IL-10, and IL-13, which are cytokines involved in a Th2 response (Figure 6). Changes in IL-2 and IL-12 secretion were not significant.

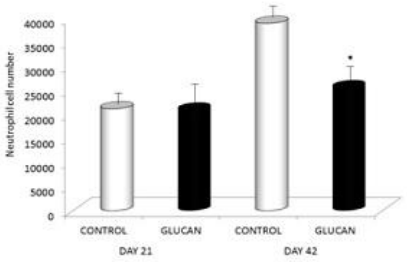


**Figure 3: Cellular infiltrates into infected joints – neutrophils. Data are expressed as mean values ± SD of three independent experiments performed in triplicates. *Represents significant differences between control (PBS) and glucan group at P ≤0.05 level.**

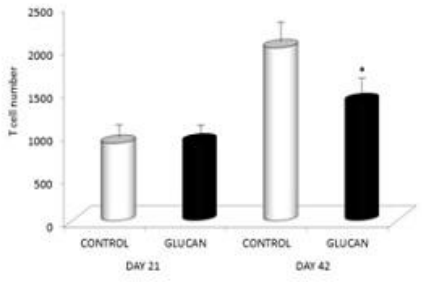


**Figure 4: Cellular infiltrates into infected joints - T lymphocytes. Data are expressed as mean values ± SD of three independent experiments performed in triplicates. *Represents significant differences between control (PBS) and glucan group at P ≤0.05 level.**

## Discussion

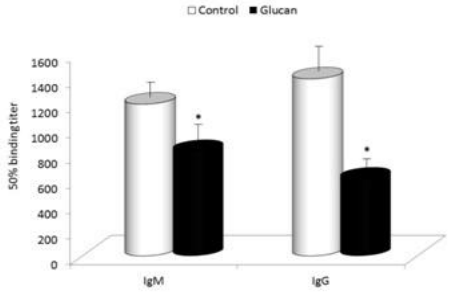


**Figure 5: Glucan supplementation lowers the titers of specific IgM and IgG antibodies. Serum was collected on day 14 post-infection. Data are expressed as mean values ± SD of three independent experiments performed in triplicates. *Represents significant differences between control (PBS) and glucan group at P ≤0.05 level.**

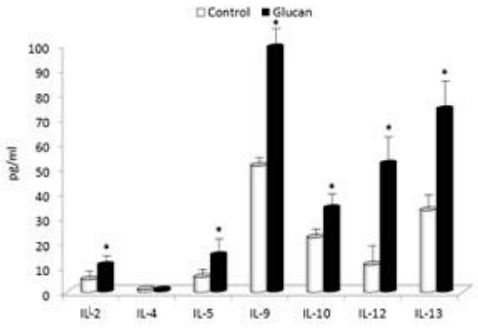


**Figure 6: Effects of glucan supplementation on cytokine production. Data are expressed as mean values ± SD of three independent experiments performed in triplicates. *Represents significant differences between control (PBS) and glucan group at P ≤0.05 level.**

We have examined the effects of glucan supplementation on some reactions associated with B. burgdorferi infection. Almost 40 years ago, β-glucan was described as a biological response modifier (BRM) that could stimulate tumor rejection in mice [13]. For a long time, β-glucan has been studied in infections. Using several experimental models, it has been well

established that β-glucan protects against infection with both bacteria and protozoa. It also enhances antibiotic efficacy in infections with antibiotic-resistant bacteria. The protective effect of β-glucan was shown in experimental infections with Candida albicans, Streptococcus suis, Plasmodium berghei, Staphylococcus aureus, and Escherichia coli; for review, see Vetvicka and Novak [2011]. Despite the fact that infections were one of the first studied actions of glucan in vertebrates, the question of glucan and parasites remains rather overlooked. Most of the studies revealed positive effects (for review, see Vetvicka and Fernandez-Botran [2018]). Glucan was found to affect biological reactions in every species tested. From these results, it is not surprising that glucan is intensively studied in humans, also [16-18]. As CR3 receptors, known to bind glucan, also mediates the binding of opsonized B. burgdorferito mammalian cells [19] and is directly involved in phagocytosis of B. burgdorferi [20], we decided to study the possible effects of a daily supplementation with glucan on known effects of B. burgdorferi infection.

Data presented in our study showed that despite having no effects on ankle swelling during the infection, glucan supplementation improved arthritis severity scores and Lyme carditis. In several infection models, less inflammation is usually associated with reduction of neutrophils recruitment. As the role of neutrophils in development of Lyme arthritis is known, we measured different subpopulations of infiltrating cells and found that glucan treatment significantly improved infiltrating cell numbers on Day 42.

A robust antibody response against B. burgdorferi is well known and clearly plays an important, despite insufficient, role in bacterial clearance. In glucan-supplemented mice, we saw a significant decrease in the titers of IgM and IgG antibodies, the two classes known to be elevated during the response against B. burgdorferi infection [21, 22]. Cytokine profile of infected mice corresponded with previously published data [12] and showed strong downregulation of Th2 response. However, it is important to note that it appears that not just Th2 but other cellular (both T and B) responses are activated after glucan ingestion. In the glucan-supplemented group, we found significant improvements of the secretion of these cytokines, which probably contributed to amelioration of Lyme disease or at least arthritis [23].

## Conclusion

From our data we conclude that food supplementation with glucan might offer a new way to suppress the effects of B. burgdorferi infection. The exact mechanisms are still unclear, but probably involve stimulation of immune reactions, particularly Th2-related cytokines.

## Learning points

Short term glucan supplementation ameliorates health problems associated with B. burgdorferi infection.

Mechanisms of action probably involve stimulation of immune reactions, particularly Th2-related cytokines.

## Conflict of Interest

The authors declare no conflict of interest.

## Disclosures

This was not previously presented in a conference and there is no funding to declare.

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