Role of $\beta$-glucan in biology of gastrointestinal tract

Vaclav Vetvicka, Luca Vannucci, and Petr Sima

1University of Louisville, Department of Pathology, Louisville, KY 40202, USA. 2Institute of Microbiology, Laboratory of Immunotherapy, Prague, Czech Republic

Glucans, despite decades of intensive research, has been used only recently in the evaluation of intestinal biology and diseases. This review is focused on comprehensive summary of the current knowledge of transport and absorption through the gastrointestinal tract, therapy of ulcerative colitis by glucan and effects of glucan on gastrointestinal tract-related tumors. Studies demonstrating the palliative effects of orally-given glucan may have significant clinical impact, since glucan can be easily added to the food. Journal of Nature and Science, 1(7):e129, 2015

Glucon | gastrointestinal tract | ulcerative colitis

Introduction

$\beta$-D-glucans (hereafter referred to as “$\beta$-glucans”) represent part of a group of physiologically active compounds generally called “biological response modifiers.” These molecules are highly conserved carbohydrates serving as structural components of cell walls of fungi, yeast, seaweed, bacteria, and some plants. Glucans, as natural molecules, are sometimes also named “pathogen-associated molecular patterns”. While none of these terms are fully accurate, since they focus only on a few effects, they are becoming common. The history of $\beta$-Glucan’s as immunomodulators is long. The first reports showing possible therapeutic effects on malignant processes can be traced to the beginning of the 18th century [1]. The real research into $\beta$-glucan’s biological and most of all immunological effects started in the 1960s. During decades of intensive research, glucans were found to significantly stimulate defense reactions against infections and cancer [2], for review see [3,4], resulting in clinical trials [5]. Also, several additional effects were later shown, including reduction of stress [6], lowering cholesterol (for review see [7]), and the suppression of toxic effects of numerous substances such as mercury [8] or aflatoxins [9].

For a long time, the effects of $\beta$-glucan on immune reactions were considered non-specific. The main reason was the lack of knowledge about the cells and receptors involved in glucan binding. The question of the binding sites was solved by elucidation of action of Dectin-1, CR3 and other glucan-specific receptors [10,11]. Predominant cell surface receptors for $\beta$-glucans expressed on the surfaces of immunocompetent cells like monocytes, free and resident macrophages, dendritic cells, natural killer (NK) cells, and polymorphonuclear leucocytes are complement receptor 3 (CR3; CD11b/CD18), $\alpha$M$\beta$2/Mac-1 and Dectin-1 [12-15].

CD11b/CD18 is a heterodimeric complex composed of the $\alpha$M chain (CD11b) and the common chain CD18. It is expressed on the surface of polymorphonuclear leucocytes, macrophages, and NK cells [16,17]. For neutrophil response to $\beta$-glucans, the cooperation with dectin-1 is necessary but $\beta$-glucan recognition by macrophages need only the presence of Dectin-1 [18].

Activation of Dectin-1 induces the clustering of the receptor by aggregates of beta-glucans. Dectin-1 mediated signaling promotes cytokine production [19-22], the generation of ROS [23,24], and phagocytosis [19]. Significant differences among plasma concentrations of glucans of various origin and binding of $\beta$-glucans by GI and GALT cells were documented [25].

The subsequent problem was lack of knowledge of intracellular signaling pathways. From these studies, the most likely candidate is Syk kinase, as this involvement of Syk kinase was confirmed on Dectin-1 receptor. The $\beta$-glucan binding is followed by phosphorylation of Dectin-1 by tyrosine kinase Src. As a result, Syk is activated and subsequently activates the card9-bcl10-Malt1 complex. Induction of several cytokines follows [20]. For more information on $\beta$-glucan related signaling, see [26].

$\beta$-Glucan was introduced into clinical practice in 1983 and since that time is still successfully used in Japan [27]. The Western world was significantly slower in recognizing $\beta$-glucan’s potential, but the number of currently running clinical trials [28,29] suggests that $\beta$-glucan might soon become an approved drug.

Transport through gastrointestinal tract

Detailed information about the transport of $\beta$-glucan through the gastrointestinal tract is still lacking. The main route for a particular antigen (and particles including bacteria) to gain access to the mucosal system and the body is through M cells in Peyer’s patches. Peyer’s patches are traditionally considered the main site, due to their intimate localization with the intestinal lumen and presence of M cells and dendritic cells. For detailed information on the role of gastrointestinal dendritic cells see [30]. The suggestion that particular $\beta$-glucans are taken up by M cells goes back to Hasmihoto’s study [31].

A detailed study of the gastrointestinal absorption of soluble $\beta$-glucans showed that the speed of transfer differs based on the type of $\beta$-glucan used, ranging from 0.5 hr to 12 hrs. Flow cytometric analysis of cells isolated from Peyer’s patches revealed that oral administration of fluorescent-labeled $\beta$-glucan resulted in the presence of the labeled material inside the cells after 24 hrs. The $\beta$-glucan was bound and internalized by intestinal epithelial cells regardless of the fact that these cells are Dectin-1 negative. As only 10% of these cells were able to ingest $\beta$-glucan, it is possible that only a special subpopulation of these cells participate in $\beta$-glucan transfer [32].

Studies using in situ intestinal perfusion and in vitro Ussing-type chamber showed significant nonlinear intestinal absorption, most probably involving some type of specialized transporting system. The possibility of multiple transport mechanisms cannot be overlooked [33].

Experiments mentioned above showed that $\beta$-glucans translocate from the gastrointestinal tract into the systemic circulation. Whether this is an active or passive process is not be fully established. However, it seems that several cell types present in the gastrointestinal tract can be involved in this transfer, suggesting that the gastrointestinal tract might serve as a reservoir for future $\beta$-glucan absorption.

IBD and causative factors

Ulcerative colitis and morbus Crohn are inflammatory bowel diseases (IBD), which are classified among auto-immune diseases. IBDs are typically accompanied by the presence of inflammation along the digestive tract. Numerous studies conclusively indicated the direct relationship of these altered physiological or directly pathological conditions of the gastrointestinal tract (GIT) to a row of other pathologies, in which obesity [34,35], diabetes, autism, and tumor diseases are the focus of scientists and clinicians.

Conflict of interest: No conflicts declared.

Corresponding Author. Vaclav Vetvicka, University of Louisville, Department of Pathology, Louisville, KY 40202, USA.

FAX: 502-852-7674. Email: Vaclav.vetvicka@louisville.edu

© 2015 by the Journal of Nature and Science (JNSCI).
The causative factors of IBD are still unknown despite intensive research. Generally, the main suspected factors are 1) genetically or epigenetically determined tendency to inflammations [36,37] and allergies [38,39]; 2) genetically or epigenetically decreased expression of mucin genes [40,41]; 3) genome-decreased-microenvironmental interactions [42]; and 4) gut dysbiosis caused some of the following problems: dysbalance in microbiome composition and activity [43,44]; intake of non-appropriate composition of nutrition (food intolerance, too many pro-inflammatory and/or not enough anti-inflammatory nutritional components [45-47]; contaminated nutrition with toxic products of environmental microbial activity, or anorganic and organic toxic external pollutants including endocrine disruptors [48-50], and mutagens generated during technological and culinary processing of foods [51,52]; and erosion of GIT structures by reactive oxygen metabolites [53].

So many possible causes are also a reason why no universal or targeted therapy of IBD may be applied. It means that is it currently impossible to select and start special causative therapy of IBD, neither to a single important factor, nor to more factors together, with the aim to attenuate inflammatory processes. We have to keep on mind that these inflammatory processes are extending within the wall of the GIT in tight vicinity of the largest immune organ of an organism, the gut associated lymphoid tissue (GALT), subsequently leading to the production of pro-inflammatory and other factors between the internal environment of the body and gut lumen [54].

The importance of gut wall permeability in IBD

All of the detrimental factors mentioned above more or less participate in influencing the intestinal permeability. The most important cause than the high permeability plays the central role in the induction of the onset and persistence of IBD is disturbance of bi-directional trafficking of various substances (ions, nutritional components, hormones, products of metabolism, cytokines and other factors) between the internal environment of the body and gut lumen [54].

The role of tight junctions in the GIT integrity

The persistence of the gut wall is ensured by tight junctions that localize a multiprotein complex (occluding molecule family, claudins, junctional adhesion molecule family, zonula occludente proteins, interleukins, growth factors, the junction adhesion molecules, and also the receptors for some viruses) [55]. Tight junctions form selective and permeable seals between adjacent epithelial and microvascular structures within the boundary between apical and basolateral junctions become leaky in IBD [57], possibly due to the changes in epithelial cells within the boundary between apical and basolateral junctions form selective and permeable seals between adjacent molecules, and also the receptor s for some viruses) [55]. Tight junctions participate in influencing the intestinal permeability. The most important role of tight junctions is to maintain the tightness of the gut epithelium, which is crucial for the selective transport of nutrients and waste products across the gut wall. Disruption of tight junctions can lead to increased permeability of the gut wall, allowing harmful compounds to enter the bloodstream and cause inflammatory responses.

Therapy of IBD by β-glucans

β-Glucans are regarded as efficient scavengers of free damaging radicals (see above mentioned causative factors of IBD) [75,76]. Positive effects of β-glucans on immunity, notably on its cellular branch, mononuclear leukocytes and macrophages are well established [77,78]. That was the reason of the interest of experts studying immunomodulative and curative properties β-glucans of plant, mushroom, yeast, and algae origin on IBD [67, for review see 79]. The first studies on the influence of β-glucans on the immune status of vertebrate and also invertebrate animals started in the 1980s. Healing effects of β-glucans have been demonstrated in invertebrates like earthworms [80], several species of horseshoe crabs [81, for review see 82], and crayfishes [83, for review see 84], fish [85-87], mice, rabbits, guinea pigs, hamsters [84,88-90] farm animals such as sheep, pigs, and cattle [91-93] and also in humans [94].

Almost at the same time it has been shown that the colonic damage caused by the dextran sulfate sodium salt in experimental animals could be cured by plant polysaccharides [95-98]. The similarity recovery of experimental colitis induced in rats by means of intracolonic administration of acetic acid was documented after a 30 day oral treatment by β-glucans (pleuram from the mushroom Pleurotus ostreatus [99]. The same therapeutic effect was confirmed after some years for P. ostreatus α-glucans [100]. Contrary to β-glucans, the role of α-glucans in immunotherapy is still little understood [for review see 101]. In a recent investigation, the same β-glucan preparation was used for the treatment of dogs suffering from artificially induced IBD [102].

Glucan transfer and absorption through the GI tract and gut wall

β-Glucans do not currently represent essential nutrients but on the other hand, they may be successfully utilized during causative treatment of IBD for strengthening of immune functions, maintaining of human body functions, and improvement of human quality of life [103]. They are resistant to digestion in GIT and require bacterial fermentation located in the large intestine [104]. Literature on the health effects of β-glucans is extensive, but the cellular and molecular mechanisms behind the reported effects still remain unsolved.

A very limited number of studies have been devoted to the elucidation of mechanisms of absorption of β-glucans in GIT. There is still no established research on whether glucans influence directly gastrointestinal mucosa or if they are transferred to the blood circulation. Rice et al. [25] showed that fungal-derived soluble glucans translocate from the GIT into the peripheral circulation. These authors have demonstrated internalization of three structurally distinct β-glucans in epithelial cells, possibly M cells, in macrophages, and in dendritic cells, which then rapidly entered the systemic circulation. Chan et al. [105] also documented uptake and internalization of β-glucan from the gut by macrophages, which subsequently circulated in the blood and released it throughout the body.

Several studies documented that orally administered β-glucans in combination with antibodies tumoricidal effects in mice during supporting antitumor therapy [106,107], increased both IgM and IgG antibody production [108], and exerted other biological effects. On the contrary, there are other controversial studies not confirming beneficial effects of orally administered β-glucans on...
immunity [e.g. 109]. Issues regarding the usability of orally administered β-glucan and its utilization by various kinds of body cells are far more complicated. Biological effects mainly depends on the molecular form of β-glucans, their physicochemical properties and their purity, or if the soluble or insoluble β-glucans are applied. These circumstances determine the fate of glucan, i.e. if it interacts with GIT cells, or immunocompetent GALT cells, and then it is distributed throughout the body [for review see 79].

From this point of view, and as practically no studies following the transfer of β-glucans through tight junctions of the gut wall into the body exist, further and detailed research of mechanisms by which β-glucans react with the proteins and other factors of tight junction complex is needed. Equally, there is the need for further clarification of the fate of β-glucan in target tissues and its effects in the internal milieu for its ultimate approval as an effective supporting drug for the treatment of IBD.

Gastrointestinal track-related tumors
Since the first direct scientific study more than forty years ago, the anticancer activity of β-glucan has been established [110]. Not surprisingly, colon or gastric cancers were no exception [111]. From the commercial point of view, two β-glucans successfully moved from laboratories to hospitals – in Japan, lentinan and schizophyllan are approved drugs since the 1980s. Lentinan (from Lentinula edodes) is a (1-3)-glucan with five 1,3 residues and two (1-6)-β-glyco pyranoside branches in side chains and molecular weight app. 400-800 kDa. Schizophyllan has similar structure and triple helix configuration with a molecular weight of 450 kDa [112]. Lentinan is mostly used in conjunction with several types of chemotherapy, including cisplatin and paclitaxel [113]. A similar conclusion can also be reached for other mushroom-derived glucans [114]. Detailed studies recommended the use of lentinan-supplemented food in patients with gastric cancer [115]. Additional clinical trials showed that lentinan prolonged survival in gastric cancer patients receiving S-1-based chemotherapy, probably due to the Th1-mediated attack on cancer cells [113]. The quality of lentinan is constantly being developed. The new version of superfine dispersed lentinan was shown to have superior effects in the treatment of advanced colorectal cancer [116].

Intravenous administration of this β-glucan in patients with gastric carcinoma resulted in enhanced production of IL-1 and TNF-α [117]. Postoperative treatment with PSK resulted in significantly longer survival of patients with gastric cancer [118]. These data were confirmed by a large randomized clinical study in patients with resected colorectal cancer [119].

In vitro studies using Maitake-derived β-glucan showed strong inhibition of human gastric cancer cell line proliferation via induction of apoptosis. The suggested mechanisms are both caspase-3-dependent and independent pathways [120]. Using an in vivo murine model, the same β-glucan significantly inhibited murine colon cancer growth by stimulating cell-mediated immunity. Maitake glucan upregulated expression of surface markers such as CD80, CD86, CD 83 and MHC II on dendritic cells and increased the production of IL-12 and TNF-α. In addition, stimulated dendritic cells activated both allogeneic and antigen-specific syngeneic T lymphocyte responses [121]. This data suggested the possibility of using β-glucan as effective adjuvant in dendritic cell vaccination.

β-Glucan isolated from mushroom Pleurotus pulmonarius was evaluated for effects on mouse model of acute colitis and on colitis-associated colon carcinogenesis. Human colorectal cancer cells were treated with β-glucan and analyzed for inflammation. In vitro treatment resulted in apoptosis induction and in modulated expression of Bcl-2, Bax and cytochrome c. In addition, NF-κB nuclear translocation and TNF-α-induced inhibitor of nuclear factor (Ik-B)α degradation were blocked [122]. In vivo, the food supplementation with β-glucan reduced formation of aberrant crypt foci and increased the number of cells undergoing apoptosis. The authors concluded that β-glucan is an effective inhibitor of colon preneoplastic processes and that these effects are mediated via modulation of proliferation, apoptosis and mucosal inflammation [79]. These findings are in agreement with data showing that lentinan prevented carcinogenesis in chronic ulcerative colitis models by inhibiting expression of P450 IA2 [123].

Conclusion
Based on the information summarized in this report, it is clear that β-glucans represent a significant potential in the suppression or treatment of several gastrointestinal problems including colitis and Crohn disease. Even when all mechanisms of action are still not fully elucidated, sufficient data exists to increase interest in the role of glucan in transfer through the gut tissue, interaction with cells present in the gut wall, and in therapeutic effects in treatment of colitis and gastrointestinal track-related tumors.

Acknowledgements
This work was supported by the institutional grant number RVO 61388971.


44. Schippa S, Conte MP. Bivalent gangliosides vaccine in combination with placebos for high-risk neuroblastoma in second or later remission. J Pharmacol Exp Ther, 193, 2005, 709-86.


84. Vetvicka V, Sima P.

87. Vetvicka V, Vannucci L, Sima P. The effects of immunostimulants, ad juvants, and vaccine carriers in...  
85. Anderson DP. Immunostimulants, ad juvants, and vaccine carriers in... 
86. Verlhac V, Gabaudan J, Obach A, Schuep W, Hole R. Influence of...  
81. Kawabata S, Muta T, Sadaa-Kiwanaga: discovery of the function of glucans on the...  
78. Volman JJ, Ramakers JD, Plat J. Dietary modulation of immune...  
91. Buddle BM, Pulford HD, Ralston M. Protective effect of glucan...  
92. Benkova M, Boroskova Z, Soltys J. Immunostimulative effects of...  

93. Verlhac V, Vannucci P. The effects of glucans on the...  
95. Rolandelli RH, Saul SH, Settle RG, Jacobs DO, et al. Comparison of...  
106. Liu RH. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. Am J Clin Nutr, 78, 2003, 517S-20S.  
111. Jeannin JF, Lagadec F, Pelletier H, Reisser D, et al. Regression...  
113. Ina K, Furuta R, Kataoka T, Kaikyawa S, et al. Lentinan prolonged...  
116. Hamaza S, Watanabe S, Ohashi M, Yagi M, et al. Efficacy of orally administered superfine dispersed lentinan (beta-1,3-glucan) for the...  

