



GUT IN FOCUS: EXTENDED ABSTRACT

Immune modulation by non-digestible and non-absorbable beta-1,3/1,6-glucan

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Chemistry of beta-1,3/1,6-glucans

Beta-glucans comprise a very diverse group of polysaccharides (even paper) in which glucose molecules – as the only building block – are linked together by beta-linkages. Only very few beta-glucan structures, notably beta-1,3/1,6-glucans, are bioactive in the sense that they interact with receptors on immune cells and elicit specific biological responses.

Beta-1,3/1,6-glucans are branched chains of glucose molecules connected by beta-1,3-glycosidic bonds, and in which the branching points are beta-1,6 linkages, as shown in the schematic diagram for the beta-glucan found in yeast.

This net-like molecular structure constitutes the inner layer of the cell wall of baker's yeast, providing mechanical strength to the cells. In live cells, the beta-1,3/1,6-glucan structure is attached to a surface layer of complex proteoglycans (mainly proteomannans) and chitin.

Many published studies on biological effects of 'beta-glucans' have been carried out with poorly defined products and created misconceptions regarding mode of action of beta-1,3/1,6-glucans. A reference product like Zymosan contains, for instance, mannose-rich proteoglycans attached to the beta-1,3/1,6-glucan structure. Since these mannose-proteins are very potent allergens and antigens, whereas the pure beta-1,3/1,6-glucan component counteracts allergy and does not elicit any antibody production against itself, it is difficult to interpret experimental results on biological effects of Zymosan. Poor chemical description holds true also for extracts from

mycelial fungi used in experimental studies. The beta-1,3/1,6-glucan component in crude fungal extracts has therefore incorrectly been held responsible for allergenic and pro-inflammatory effects of such extracts and of molds.

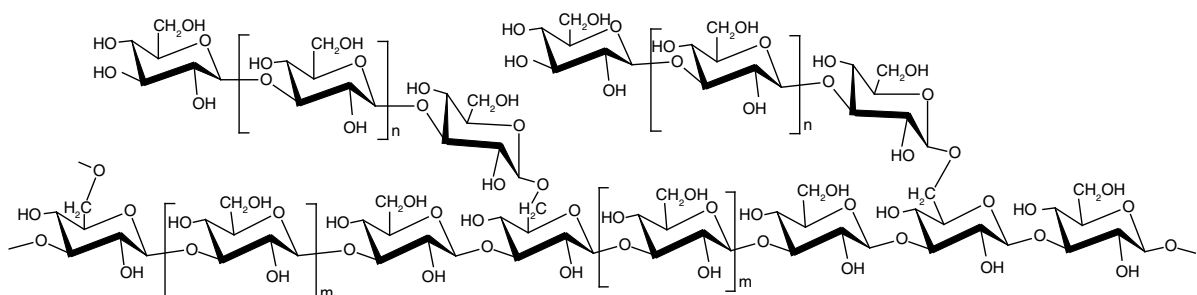
The ability of beta-1,3/1,6-glucans to activate innate immune cells depends on its branched structure. A beta-1,3-glucan without any side chains (branches) does not activate macrophages. Products with only one single glucose molecule in the 'side chain', as in most mushroom beta-1,3/1,6-glucans (e.g. lentinan), have lower macrophage activating activity than yeast cell-wall beta-1,3/1,6-glucan.

In addition to chain length, the frequency of side chains is essential for immune-stimulating and immune-modulating ability of beta-1,3/1,6-glucans (1, 2). These molecular structures may change or be destroyed during extraction, hence affecting the biological activity of the extracted product. The materials section of papers on biological effects of 'beta-glucans' should therefore be studied carefully.

Biological effects of beta-1,3/1,6-glucans

More than 30 years ago, Seljelid and co-workers screened a large number of glucans and glycans (polysaccharides containing also other sugars than glucose) for their macrophage-activating ability *in vitro*, and found that a particulate beta-1,3/1,6-glucan prepared from baker's yeast was the most active (3).

In the mid-1980s, it was first discovered in a practical field experiment that the same preparation Seljelid (3) had tested, enhanced resistance of salmon to infectious



disease (vibriosis), even when it was incorporated in the feed at a low inclusion level (0.1% on dry weight basis). The discovery that a non-digestible feed component (fish or warm-blooded animals do not produce beta-1,3- or beta-1,6-glucanases) enhanced disease resistance was not in line with textbook teaching at the time. The discovery had to be scrutinized in a comprehensive research program before it could be considered trustworthy. But before scientific studies had fully confirmed and widened the scope of the initial field observation, the feed industry took the lead and introduced the same beta-1,3/1,6-glucan product (MacroGard) as an immune enhancer in animal feeds – worldwide.

During the last 25 years, this yeast beta-1,3/1,6-glucan preparation has been introduced as a feed additive to improve health and performance of farmed shrimp, fish, pigs, chicken, laying hens and calves, and of horses and pet animals. References (4–13) are a short ‘short list’ of experimental studies and reviews supporting the rationale of such use.

Mode of action of beta-1,3/1,6-glucans

The beta-1,3/1,6-glucan interacts with specific receptors (dectin-1, TLR 2/6, CR3) on white blood cells in the innate immune system (14–17), such as macrophages, neutrophils, granulocytes, natural killer cells, dendritic cells, and corresponding cells found in tissue surfaces.

Mucosal/oral administration of beta-1,3/1,6-glucans initiate biochemical processes leading to enhanced infection defense (4–13), enhanced antibody production against mucosal antigens (18), enhanced efficacy of injected monoclonal cancer antibodies (19), faster regeneration of physically damaged tissues (20), enhanced healing of (diabetic) wounds (21), reduced toxicity of bacterial endotoxin (22), and reduced gut infections (23, 24).

It is difficult to find one unifying, mechanistic model of mode of action 1,3/1,6-glucan at the cellular level explaining all of these effects. It is not clear to what extent the substance is taken up from the gut when it exerts its effects. Small amounts of beta-1,3/1,6-glucan may be taken up into endothelial cells, and the quantity in other tissues has always been found to be extremely low (25). It seems therefore most likely that the primary action of beta-1,3/1,6-glucan is in the gut epithelia and that systemic effects are secondary results of this interaction.

Anti-inflammatory effects of beta-1,3/1,6-glucans

Numerous farm reports have consistently referred to observations that orally administrated particulate beta-1,3/1,6-glucans have anti-inflammatory effects in animals, in addition to enhancing infection defense, in particular in settings where there is a high infection load. Commercial field trials have, for instance, confirmed that beta-1,3/1,6-glucan used as supplement to feeds reduces gastro-

enteritis problems in chicken and improve their general performance and increase egg yield in laying hens.

These positive effects may be the result of enhanced activity of intestinal immune cells (26), improved intestinal barrier function (27), stimulated formation of intestinal immune cells (23, 28), and reduced LPS-induced toxicity (22).

Already in the 1980s, the group of Rolf Seljelid (29) showed that injection of a pure beta-1,3/1,6-glucan derivative into mice rendered the animals very resistant to injected *Escherichia coli* and to endotoxemia. Later studies have shown that a highly purified and soluble version of the particulate beta-1,3/1,6-glucan used in animal feeds protects against lipopolysaccharide (LPS)-induced shock in rats (22). These studies demonstrated that the protective effect of orally administered beta-1,3/1,6-glucan was better than that of injected product. The serum concentration of beta-1,3/1,6-glucan was very low (3 ng/ml) in animals given the product orally and less than 1/40 of the concentration in animals given it by injection. In the experimental model study, rats were given beta-1,3/1,6-glucan (20 mg/kg body weight/day) for 14 days orally before they were subjected to endotoxemia by intravenous infusion of *Escherichia coli* LPS (6 mg LPS/kg). Rats pretreated with oral beta-1,3/1,6-glucan recovered significantly faster from LPS-induced blood pressure collapse than rats in the control group and faster than in rats pretreated by injected beta-1,3/1,6-glucan. Oral pre-treatment also significantly attenuated LPS-induced rise in plasma creatinine, aspartate aminotransferase and alanine aminotransferase, indicating protection also against LPS-induced renal and hepatic injury.

A US-patent from 2009 (23) describes how the same beta-1,3/1,6-glucan product (as in 21) can be used as an oral treatment and prevention of inflammatory diseases in the intestinal tract. A pure mushroom beta-1,3/1,6-glucan (lentinan) has later been shown to have corresponding effects (24), even at the same low concentration. Both groups have shown that oral administration of beta-1,3/1,6-glucan exhibits intestinal anti-inflammatory activity, and they suggest that beta-1,3/1,6-glucan may be effective for the treatment of gut inflammation, including inflammatory bowel disease (IBD).

Beta-1,3/1,6-glucans counteract not only LPS-induced inflammations but also the inflammation elicited by influenza virus (18). Nasal administration of particulate beta-1,3/1,6-glucan prior to intra-nasal infection by the virus, remarkably reduced disease score resulting from the cytokine storm elicited by the influenza virus.

The ability of beta-1,3/1,6-glucan to suppress inflammatory response has been tested also in humans scheduled for coronary artery bypass grafting (20). Pretreatment for 5 days with oral particulate beta-1,3/1,6-glucan caused significantly lowered creatine kinase isozyme

and cardiac troponin levels the first day post operation, and it was concluded that beta-1,3/1,6-glucan pretreatment is safe and *may protect against ischemia reperfusion injury following CABG*.

Beta-1,3/1,6-glucan and gut microbiota

The biological effects beta-1,3/1,6-glucans are usually explained within the framework of conventional immunology, as the result of the interaction between beta-1,3/1,6-glucan and specific receptors on epithelial surfaces (24). However, there are effects which may involve the gut microbiota, such as suppressive effects of pure beta-1,3/1,6-glucan on asthma and allergy symptoms.

Beta-1,3/1,6-glucan molecules will pass non-digested through the small intestine, but become the substrate for microbes in the colon. The amount of pure beta-1,3/1,6-glucan exerting significant biological effects is low, however, and it is therefore unlikely that it will be a significant energy substrate for microbes living under anaerobic conditions in the colon. Beta-1,3/1,6-glucan may nevertheless interact indirectly with the gut microbiota by affecting intestinal barrier function and LPS toxicity, and by enhancing the production and secretion of components such as lysozyme, antimicrobial peptides and IgA.

Immune modulating, pure beta-1,3/1,6-glucans exert their effects at very low concentration in the diet, and should therefore not be compared with the 'fiber effects' of dietary cereal beta-glucans. The latter are different in chemical composition and they affect gut functions and gut microbiota at much higher concentrations.

There are unreported observations on the effects of pure immune modulating beta-1,3/1,6-glucans on the composition of the gut microbiome of warm-blooded animals, but such studies have not been given priority in scientific follow-up studies – yet. But within the fish farming sector it is different (30).

Perspectives

Many studies with poorly described, crude beta-glucan preparations have contributed to confusion, misconceptions, and controversies regarding biological effects of chemically well-defined beta-1,3/1,6-glucans. Beta-1,3/1,6-glucans are already attractive pharmaceutical products and product candidates for a number of clinical indications.

Due to their ability to enhance infection defense mechanisms and simultaneously down-regulate inflammations, beta-1,3/1,6-glucan is very promising as an alternative to the mainstream use of immunosuppressive drugs to treat inflammatory diseases, for instance, IBD. New formulations – not necessarily based on beta-1,3/1,6-glucans alone – will certainly be developed and used prophylactically and possibly also therapeutically to reduce the need for antibiotics in human and veterinary medicine.

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