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Minireview

β-Glucan and parasites

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Summary

Immunosuppression caused by parasitic infections represents the foremost way by which the parasites overcome or escape the host's immune response. Glucan is a well-established natural immunomodulator with the ability to significantly improve immune system, from innate immunity to both branches of specific immunity. Our review is focused on the possible role of glucan's action in antiparasite therapies and vaccine strategies. We concluded that the established action of glucan opens a new window in treatment and protection against parasitic infections.
Keywords: glucan; parasite; Toxoplasma; Leishmania; immunity

Background

Natural products that are useful in treating various diseases have been intensively sought after throughout the history of mankind. Almost than 40 years ago, β-glucan was described as biological response modifier (BRM) that could stimulate tumor rejection in mice (Yanagawa *et al.,* 1984). As with many other BRM, it was classified as "nonspecific" because the cellular and molecular targets were unknown and its effects appeared to be highly pleiotropic and even more unpredictable. Despite long-term interest and research, the mechanism of how β-glucan affects various biological processes remained an enigma for a rather long time. Only in the last decade has extensive research by numerous scientific groups helped to reveal the extraordinary effects that β-glucan has on our immune system. A schematic representation of the basic molecular structure of β-glucan is presented in Figure 1.

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, and 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).

Among the well-studied pleiotropic effects of β-glucan, we can mention stimulation of both humoral and cellular immunity (Novak & Vetvicka, 2008), metabolic control of diabetes (Wursch & Pi-Sunyer, 1997), stimulation of wound healing (Browder *et al.*, 1988), stress reduction (Vetvicka & Vetvickova, 2014), attenuation of chronic fatigue syndrome (Vetvicka & Vetvickova, 2015), lowering cholesterol levels (Braaten *et al.*, 1994), and inhibition of cancer (Sima *et al.*, 2015). Readers seeking a summary of glucan actions can read a recent monography by Větvička (2013) or other additional excellent reviews (de Oliveira Silva *et al.*, 2017; Vannucci *et al.*, 2017; Bacha *et al.*, 2017; Vetvicka *et al.*, 2017; Alves da Cunha *et al.*, 2017). A schematic view on the role of glucan in stimulation of immune reactions is shown in Figure 2. For more information on innate and adaptive immunity, see Netea *et al.* (2016).

Another advantage of glucan use is that it works in many species. Glucan has been found to be active in invertebrates, including

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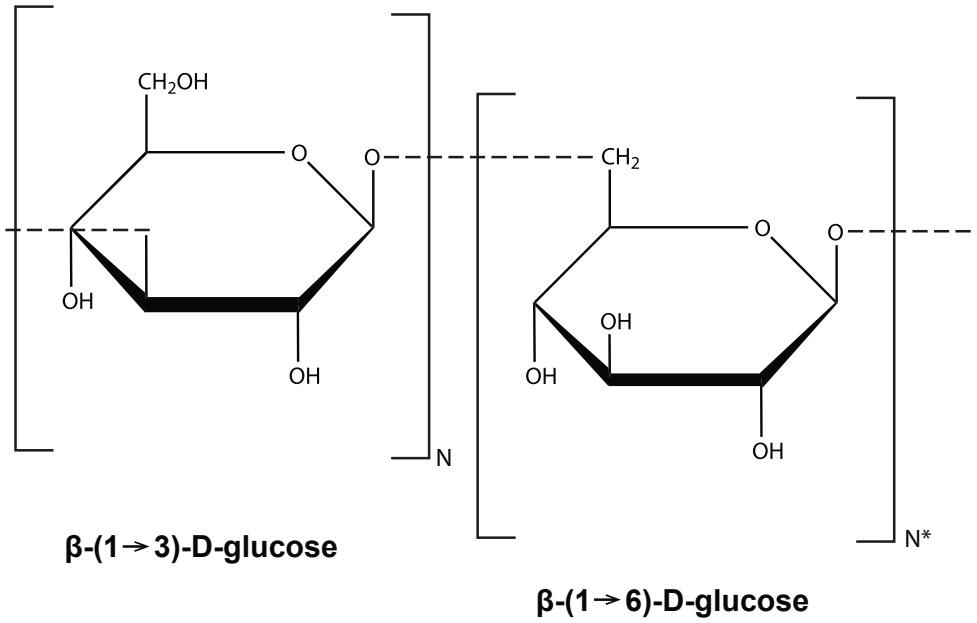

Fig. 1. Schematic representation of the basic molecular structure of glucan molecule.

earthworms (Beschin *et al.*, 1998), bees (Mazzei *et al.*, 2016), and shrimp (Duvic & Soderhall, 1990), and in vertebrates, including fish (Anderson, 1992), chickens (Vetvicka & Oliveira, 2014c), mice (Patchen & MacVittie, 1982), rats (Horvathova *et al.*, 2008), hamsters (Wang *et al.*, 1997), dogs (Vetvicka & Oliveira, 2014b), pigs (Vetvicka & Oliveira, 2014a), calves (Buddle *et al.*, 1988), and monkeys (Reynolds *et al.*, 1980), possibly making it the only immunomodulator active in every species tested. It is clear that with so many reports; several types of glucan were used, leading to the question if the same glucan will have the same results across species. So far only one study exists directly comparing effects of two different types of glucan in chicken, mice, dogs and pigs (De Oliveira *et al*, submitted). The study showed that these glucans had identical effects in all four different species. From these results, it is not surprising that glucan is intensively studied in humans, too (Kushner *et al.*, 2014; Richter *et al.*, 2014; Větvička, 2013).

Special role of glucan action has been established in invertebrates, representing one of the major defensive mechanisms. Glucan is involved in the prophenoloxidase system, and glucan-binding protein with a specific affinity to glucan plays an important role in protection of invertebrate animals, especially arthropods, against parasites and other invading pathogens; for review, see Vetvicka and Sima (2017) and Soderhall and Cerenius (1998).

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. At the same time, parasitic diseases are a major cause of morbidity and mortality, with more than three billion people infected worldwide (Bhutta *et al.*, 2014, Torgeson *et al.*, 2015). As most infections occur in developing countries, the need for a dependable and economical cure/prevention is particularly high. As the immune system of infected individuals seems to be particularly compromised (Samuel, 2016), an established immunostimulant, such as glucan, might be the ideal solution. The role of glucan in parasitic infection was intensively studied in the 1980s and, after three decades of neglect, the focus of glucan studies is slowly returning to this topic.

Mechanisms involved in glucan stimulation of immunological and inflammatory responses fall beyond the scope of this review. However, the most important action resulting in adequate stimulation is probably the way glucan interact with their receptors. The main glucan receptors are complement receptor 3 (CR3, CD11b/CD18) and Dectin-1. The first receptor belongs to the β_2-integrin family and is found mostly on macrophages, leukocytes, and NK cells. Glucan bind to the lectin site of this receptor and the overlapping I-domain of CD11b. The stimulation of cells relies on simultaneous binding of glucan and iC3b-opsonized material (Xia *et al.*, 1999). On the other hand, Dectin-1 is a type II transmembrane protein present on neutrophils, macrophages and dendritic cells. Upon binding of glucan, an immunoreceptor tyrosine-based activating motif is phosphorylated (Brown, 2006). In addition, stimulation of Dectin-1 receptor by glucan is mediated via Syk/NF-kB signaling axis (Fang *et al.*, 2012). For a review dedicated to the molecular interaction of glucan with receptors, see (Legentil *et al.*, 2015). The confusion regarding the effects of various route of administration was finally resolved by studies carefully comparing the effects after individual routs of administration and showing that the effects are the same (Vetvicka & Vetvickova, 2008; Vojtek *et al.,* 2017).

The development of an entirely new class of antiparasitic drugs is rare and lately seems to be near impossible. At the same time, parasitic pathogens, particularly the intracellular pathogens, are as

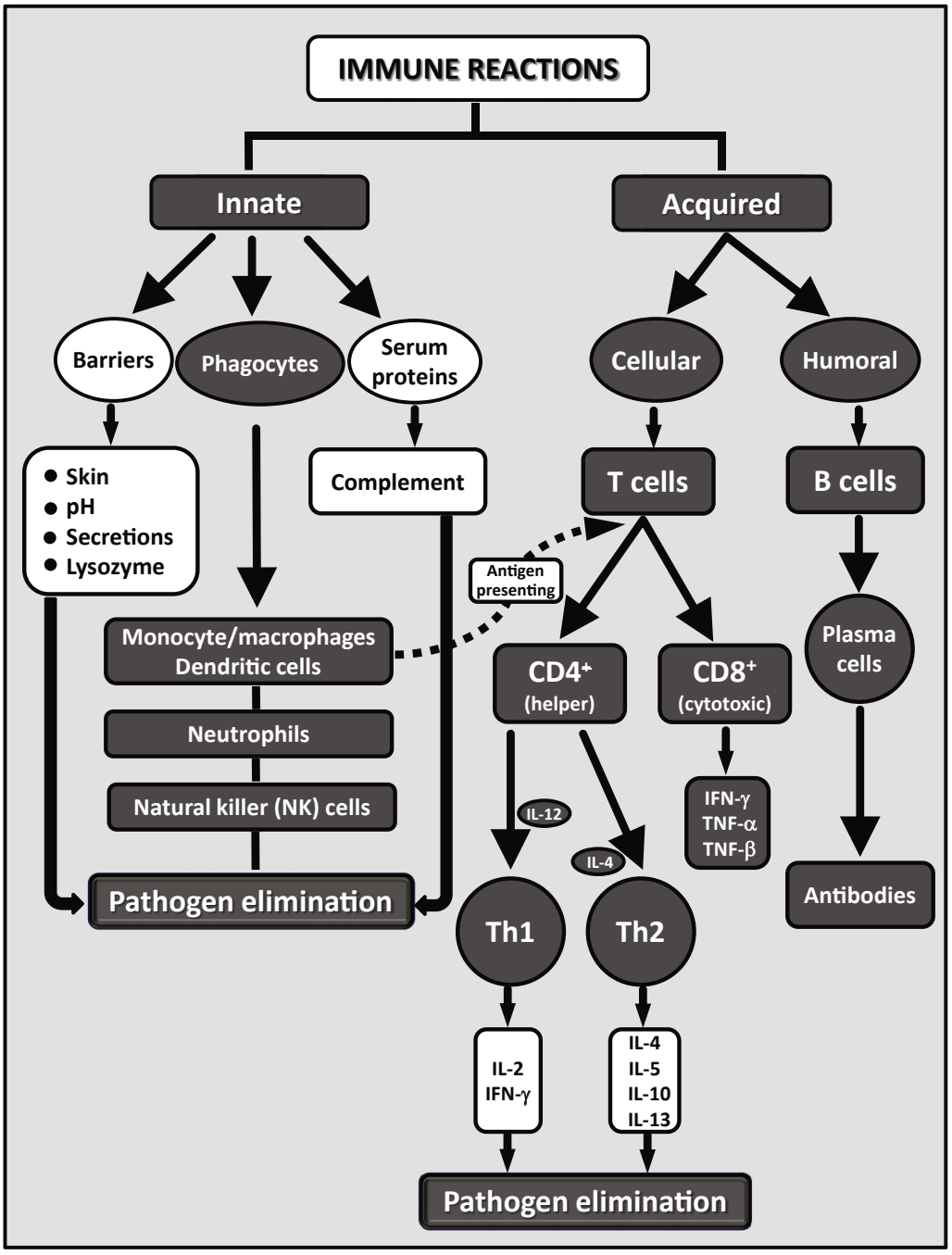

Fig. 2. Various aspects of the both branches of immune reactions. Reaction known to be influenced by glucan are represented in black, reactions where glucan has no confirmed effects are shown in white.

dangerous as ever. The question is – can glucan satisfy the need for a new drug?

Leishmania

One of the most studied parasites is *Leishmania*. Under normal conditions, the immune system cannot cope with this infection, so it is necessary to significantly boost immune reactions. An *in vivo* experiment used a genetically susceptible mouse strain infected with *Leishmania major* (Goldman & Jaffe, 1991) and showed that four intravenous injections of glucan resulted in significant suppression of infection, whereas intraperitoneal injections produced little or no effects. Older experiments showed not only protection using combination of glucan and killed *Leishmania*, but also the

positive effects of adoptive transfer by spleen cells isolated from vaccinated animals (Jarecki-Black *et al.*, 1985). Subsequent observation showed that four intraperitoneal injections prior to infection with *Leishmania* offered significant reduction in the amastigote proliferation (Al Tuwaijri *et al.*, 1987).

In an *in vitro* experimental design, infected J-774A.1 macrophages stimulated with glucan showed elevated amounts of host-protective molecules such as nitric oxide and inflammatory cytokines. Even more interesting was the synergy of glucan with a standard drug, miltefosine (Shivahare *et al.*, 2016).

Another interesting approach demonstrating the effectiveness of glucan was its use in an experimental vaccine based on *L. donovani* promastigotes. This vaccine offered significant protection regardless the route of application (Cook & Holbrook, 1983; Novak & Vetvicka, 2008). These data were based on older studies showing good effects of intravenous glucan injections and even stronger stimulation by glucan-promatostigotes combination (Cook & Holbrook, 1983). Similar data were obtained using a hamster model, where besides the effects of a glucan-prostigmatoses combination, significant protection was found after application of glucan alone, both *in vivo* and *in vitro* (Cook *et al.*, 1982). The resistance caused by injection of glucan lasted up to 80 days (Holbrook *et al.*, 1981b). Glucan alone offered protective effects in combination with every antigen fraction tested (Obaid *et al.*, 1989). Similar results were obtained on a model of *L. infantum* (Lasarow *et al.*, 1992). Glucan offered protection against *L. amazonensis* via stimulation of NK cell activities (Yatawara *et al.*, 2009). It is important to note that whereas different authors used different routes of glucan administration, the effects of glucan were same application (Cook & Holbrook, 1983; Novak & Vetvicka, 2008), which further supports the fact that glucan is active via all ways of administration.

Another study found that a 45-day application of glucan eliminated the spleen and liver parasite burden in a model of visceral leishmaniasis. Detailed analysis suggested the importance of glucan-mediated production of interleukin-12 and interleukin-17 (Ghosh *et al.*, 2013).

Other infections

A similar study evaluating the synergy between antihelmintic drug praziquantel and glucan used mice infected with *Mesocestoids vogae* tetrathyridia. The results showed that combined treatment resulted in suppression of fibrogenesis in the liver cell protection against oxidative damage, and possible stimulation of parenchyma regeneration (Velebny *et al.*, 2008). The same group previously reported that a synergistic therapy with glucan and praziquantel increased macrophage activity and resulted in increased immunoglobulin levels to the secretory antigens, but decrease to the somatic antigens, probably caused by changes in antigen exposure (Hrckova *et al.*, 2007).

Toxoplasma gondii is a common intracellular parasite, particularly dangerous for immunocompromised individuals, as the infection results in suppression of cell-mediated branch of immune reactions. The use of glucan stimulated the production of interleukin-10 in infected animals more than the standard drug, sulfadiazine (Picka *et al.*, 2005), but the relevance to the potential treatment is unclear, as the study did not measure the possible changes in parasitic load.

Older studies showed 100 % protection against formalin-killed erythrocytic stages of *Plasmodium berghei* after simultaneous intravenous glucan injections (Holbrook *et al.*, 1981a). Mushroom-derived lentinan was used during blood-stage infection with *Plasmodium yoelii*. When used as a prophylaxis, glucan strongly decreased parasitemia and increased overall survival rate. Stimulation of Th1 (subset of T helper lymphocytes) response was suggested due to the stimulation of nitric oxide, interleukin-12, and interferon-γ production. In addition, this study found stimulation of dendritic cell maturation and reduced Treg (regulatory T lymphocytes) action (Zhou *et al.*, 2009). As this group used lentinan, glucan already approved for clinical use, the results have strong clinical potential.

In a study of *Eimeria vermiformis* infection, mice were first immunosuppressed with dexamethasone, then infected with oocysts of *E. vermiformis*, and finally treated with oat β-glucan by intragastric or subcutaneous routes (Yun *et al.*, 1997). Fecal oocyst shedding was reduced in the glucan-treated groups compared to the control group. Immunosuppressed mice which received no glucan treatment showed more severe clinical signs of the disease and a 50 % mortality, while minimal clinical signs and no mortality were recorded in the glucan-treated groups. In addition, all classes of immunoglobulin showed elevated levels. Yun *et al.* (2003) later showed that oat glucan lowered fecal oocyst shedding by 40 %, probably via changes of lymphocyte populations in various lymphatic organs.

In aquaculture, glucan represents an important part of the supplements-driven stimulation of immune system. As infection with parasitic ciliate *Ichthyiohthirius multifiliis* is often fatal, it is not surprising that glucan-supplemented food was tested as possible protection. Comparing short- and long-term applications, the study confirmed the need for longer administration of glucan (Lauridsen & Buchmann, 2010). A similar study showed significant protection after longer application (Jaafar *et al.*, 2011). In addition, glucan offered protection against *Loma salmonae* given either intraperitoneally or orally (Guselle *et al.*, 2010), offering a new window for successful vaccination of commercially farmed fish. Later studies using common carp, however, did not confirm these results (Herczeg *et al.*, 2017). For a summary on glucan-derived stimulation of immune reaction in case of *L. salmonae* infection, see Rodriguez-Tovar *et al.* (2011).

Glucan combined with zinc and porcine immunoglobulins significantly reduced the number of larvae in *Toxocara canis* infections (Soltys *et al.*, 1996). As the authors never explained the reasons behind this particular combination, the results are difficult to interpret. Later experiments showed that glucan alone, when applied

with a highly infective dose of *T. canis* eggs, showed strong stimulative and restoration effects (Boroskova *et al.*, 1998).

Other aspects

Besides stimulating antiparasitic immunity and subsequently suppressing the parasitic infection, glucan can also be involved in a completely different role. In addition to helping to protect the host, glucan can also play a direct role in life of the parasite. Glucan is present in oocyst walls of *Toxoplasma* and *Eimeria*, where its fibers are part of trabecular scaffold in the inner layer of oocyst wall, but it is not a component of sporocyst and tissue cyst walls. This glucan might be targeted by drugs specific for glucan synthase (Bushkin *et al.*, 2012). However, the absence of glucan in tissue cysts suggests that glucan receptors are not involved in human innate and acquired immune responses to *Toxoplasma*.
In the case of the intracellular pathogen *Histoplasma capsulatum*, the binding of the pathogen to the membrane of macrophages is mediated by the glucan present in the cell walls. In addition, alpha glucan is important for *H. capsulatum* virulence; whereas, glucan is antigenic and are involved in modulation of the host immune response (Gorocica *et al.*, 2009). Unfortunately, no additional information is currently available.

Conclusion

In two waves of scientific interest, spanning three decades, glucan studies have consistently shown its ability to offer solid protection against parasitic infections. Despite positive effects, glucan treatment has, in general, been considered questionable, particularly due to the problems with obtaining the same glucan in subsequent batches (which is inherited problem to most natural molecules), and to the lack of knowledge of the mechanisms of action. Some of the confusing results originally reported might be contributed to the lack of high-quality glucan available at the time of those studies.
The overwhelming conclusion reached from this review is that, as an adjuvant, glucan can be as effective as, and at the same time safer than, conventional bacterial or other adjuvants (Roohvand *et al.*, 2017; Li & Wang, 2015; De Smet *et al.*, 2014). In the last decade, our knowledge of glucan and its mechanisms of action have improved tremendously. In addition, large companies are now able to produce large batches of glucan, allowing the researchers to work with identical glucan for many years. Our short review described the current knowledge of glucan action in various parasitic infections. We believe that glucan application might open a new window in treatment and protection against parasitic infections via development of vaccines.

Conflict of interest

Authors declare no conflict of interest.

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