REVIEW ARTICLE

β-Glucans as Immunomodulators

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ABSTRACT

While polysaccharides have been used for decades in several countries to fight cancer and to stimulate the immune system, only in recent years have they become a focus of intensive studies. Despite numerous studies, we are just beginning to understand the precise mechanisms of glucan-cell interactions. The following text represents a short review of the most important observations in this interesting and potentially both commercially and clinically important field.

INTRODUCTION

Polysaccharides have a long history as immunomodulators. The first investigation of the tumor-necrosing effects of the so called Shear's polysaccharide was published almost 60 years ago.[1] Interest in polysaccharides rose after experiments showed that zymosan (crude yeast cell preparation) stimulates macrophages via activation of the complement system.[2-4] After that, the focus of numerous investigators was on glucans. Glucans are glucose polymers that occur as a primary component in the cell wall of fungi and bacteria. In addition, they are often secreted extracelluarly by various fungi.[5] Numerous studies have shown that β-glucans, either particulate or soluble, exhibit both antibacterial and antitumor activities.[6,7]

Around 1985, two types of glucan, lentinan and schizophyllan, were licensed in Japan as immunostimulants effective in cancer therapy. Lentinan is obtained from the edible mushroom *Lentinus edodes*; schizophyllan is isolated from the cultural fluid of *Shizophyllium commune*. Both glucans are branched 1,3-β-glucans. Using a model of Sarcoma 180 tumors grown in mice, both glucans were shown to have strong tumor-inhibiting activity.[8-10] A comprehensive review of immunomodulatory effects of lentinan is given by Chihara.[11]

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DIFFERENT SOURCES OF GLUCAN

Various types of glucans can be isolated from almost every species of yeast. However, glucan derived from *Saccharomyces cerevisiae* (baker's yeast) has been the most extensively studied and has produced the highest biological effects. Glucan forms part of the yeast cell wall, together with mannan, proteins, lipids, and small amounts of chitin.

The isolation of glucans from various types of mushrooms was a logical follow-up of the folk remedy use of mushrooms in many nations. Readers seeking review of different isolation processes and biological effects should see the review by Miski and Kalenta.[10] Besides the differences in structure, these glucans differ widely in their branching and 3D structure. Their immunostimulating activity is associated with the triple-helix conformation of the backbone. Addition of D-arabinofuranosyl or D-mannopyranosyl branches increased the antitumor activity of these glucans.

IMMUNOMODULATION

The immunomodulating effects of β-glucan are well established during the development of immune reactions. The stimulative effects of various glucans have been described in various evolutionary "lower" creatures such as arthropods.[12,13] The mechanism of action is the activation of the clotting system through a cascade of reactions based on several serine proteinases. The end point of this cascade is the formation of a gel that immobilizes the bacteria and

us prevents its spread. Similarly, a β-glucan-binding pro-in is an important factor in boosting the immune system crustaceans such as lobsters and shrimps. This protein hances the activity of the prophenoloxidase-activating zyme, which then induces the activation of phenoloxi-se.[14,15] In addition, glucan also directly activates the rimp hemocytes.[16] Subsequent studies demonstrated that glucan has strong immunostimulating activity in other ecies, including earthworms,[17] fish,[18] chicken, mice, ts,[19] rabbits, guinea pigs,[20] sheep, pigs,[21] and cattle.[22,23] ased on these results, we can conclude that β-glucan mmunostimutaion is active over a broad spectrum of bio-gical species and is one of the first immunostimulants tive across the evolutionary spectrum. β-glucan is a bio-gically active polysaccharide considered to be an evolu-nary extremely old stimulant of defense reactions of all nds. Clearly, the recognition of β-glucans as major com-nents of yeast, bacterial, and fungi cell walls belongs to e first defense mechanisms that evolved during phyloge-tic processes.

The number of individual glucans is almost as great as e number of sources used for its isolation. Despite numer-us studies performed all over the world, it is impossible to y that only one particular glucan is the optimal mmunomodulator. Different physicochemical parameters, ch as solubility, primary structure, molecular weight, anching, and polymer charge also play a role in deter-ining whether the polysaccharide modulates immune actions. From Yadome's[24] recent review on the structur-properties of glucans affecting biological activities, one n draw some conclusion. Branched or linear 1,4-β-glu-ns have very limited, if any, activity. Glucans with a 1,6 nfiguration usually have limited activity. β-glucans with 1,3 configuration with additional branching at the position -6 of the 1-3 linked D-glucose residues have the highest mmunostimulating activity.

The pharmacokinetic parameters of β-glucan are xtremely important for calculating therapeutic dose levels. lthough larger glucans appear to be better because of their sistance to glomerular filtration and saturation of liver learance, glucans that are too large have a higher potential r producing undesirable side effects. This condition is pecially important when low molecular soluble glucans e used, as these glucans bind to individual CR3 molecules nd prime these receptors to react only when later cross-nked by iC3b-coated prey.[25] Here we can talk about a ghly specific stimulation of one particular mechanism of e whole cascade of immune reactions.

In contrast to the water soluble low molecular weight -glucans, β-glucans that are either large enough or insolu-le can activate immune reactions nonspecifically, in bsence of a specific target moiety. Various large glucans ch as SSG, schizophyllan, or lentinan trigger a respirato-y burst and stimulate the production of various immunoac-tive substances such as IL-6, IFN, and TNF.[26-30] In addition to their effective use via intraperitoneal and intravenous injection, glucans remain active even when given orally.[32,32]

Despite all the research, cellular and molecular mech-anisms responsible for glucan effects on the immune sys-tem are not completely undestood. The first step is binding to specific receptors present on various cell types involved in defense reactions. In addition to the specific priming of these receptors, the less specific or even entirely nonspecif-ic stimulation of immune reactions occurs via subsequent release of biologically active molecules such as interleukin 6, nuclear factor βB, superoxide anion, and tumor necrosis factor.[33] The binding of β-glucans to the specific β-glucan receptor on macrophages and neutrophils is well docu-mented.[34-37] The binding results in Ca^{2+} influx through receptor-operated channels, regulated by protein kinase C.[38] Anti-infective properties of yeast-derived β-glucans are summarized in a comprehensive review by Bleicher and Mackin.[39] Like with most, if not all, effects of glucans, these properties are again due to the stimulation of macrophages and their microbicidal potential.

Detailed analysis of the interaction of human cells with β-glucans has demonstrated that complement receptor type 3 (CR3; CD11b/CD18) is primarily responsible for both the binding and biological effects of β-glucans. CR3 is consid-ered to be the most important receptor mediating the clear-ance of iC3b-opsonized immune complexes by the phago-cytic system. In addition to being a receptor for cytotoxici-ty and phagocytosis, it also serves as an adhesion molecule responsible for leukocyte diapedesis. The mechanism of the activation of CR3 by β-glucan is initiated by the binding of β-glucan to a lectin site in a CR3 molecule. After the bind-ing, the CR3 is primed for cytotoxic degranulation in response to the binding of an iC3b fragment to a different part of the CR3 molecule.[28,37,40] These data were further validated by the use of cells from CR3-deficient mice, which were resistant to the β-glucan effects.[41]

EFFECTS ON HUMANS

The overwhelming majority of experimental data has been obtained from various animal models; only limited information about immunostimulation of the human system is available. Browder et al described stimulation of human macrophages in trauma patients.[42] Glucan therapy strongly decreased septic morbidity. A multicenter, double-blind study found an optimal dosage of glucan in high-risk surgi-cal patients. In addition, these studies demonstrated the safety and efficacy of glucan in surgical patients who underwent major thoracic or abdominal surgery. As no adverse drug experiences associated with glucan infusion have been found, glucan-treated patients had significantly lower infections.[43,44] Lentinan has been shown to increase the numbers of CD4 positive lymphocytes in lymph nodes

of gastric cancer patients. In addition, the numbers of CD4, Leu 11 (NK cells), and LeuM3 (macrophages) cells among umor-infiltrating lymphocytes increased.[45] In a different study, the lifespan prolongation effects of lentinan in combination with chemotherapeutic agents had been confirmed n patients with either inoperable or recurrent gastric cancer.[46] Positive effects were also found in patients after cardiopulmonary bypass.[47] Surprising inhibition of antiviral activity has been found in HIV-infected patients.[48] A soluble yeast-derived glucan called Betafectin showed promising results both in volunteers, preclinical animal studies, nd in Phase I/II clinical trials, but for unknown reasons, the inal 30-centre, 1,236 patients Phase III trials failed. In ddition to immunomodulatory effects, β-glucans were also hown to reduce the total and LDL cholesterol levels of ypercholesterolemic patients.[49]

'OXICOLOGY OF GLUCAN

Despite numerous studies on the biological effects of lucan, complete toxicology is still not fully established. 'he acute and subacute toxicities of lentinan were examned by intravenous injection into mice, rats, dogs, and ionkeys. In all experiments, the LD_{50} was more than 100 ig/kg. Using lower doses, no chronic toxicity was oserved. In addition, no hypersensitivity was found in anaıylaxis tests.[11] A 52-week oral toxicity study of a *Candida 'bicans*-derived β-glucan revealed no toxicity and no side 'fects up to the highest tested dose of 200 mg/kg/day. The uean-fed rats showed no physiological differences from e control groups.[19]

JMMARY

Investigations of substances nonspecifically affecting ımune reactions are becoming more and more important om the view of potential clinical practice and from the ew of veterinary medicine. More and more commercially ıportant animals are farmed under stressful conditions, d the ability to boost their immunity by easily prepared mmercial immunostimulants will strongly decrease mority induced by stress-related illnesses. In animals, glucns are being tested not only as nonspecific stimulants, but o as an addition to currently used vaccinations.[23] In addin to veterinary and commercial practices, immunomoduory polysaccharides are currently the focus of intensive eclinical and clinical investigations on several parts of the obe. The question "which glucan should be picked as the timal immunomodulator?" remains open. However, one nclusion can be made — the yeast-derived glucans have advantage of being a cheaper and a more defined source well as having higher biological activity. Unfortunately, 'eral commercial sources of β-glucan offer inferior prod-, derived either from crude extracts or primarily from cose or mannose. Tests performed in our laboratory showed that the majority of commercially available glucans have very limited biological activity. Some new companies, however, are devoting particular attention not only to isolation and characterization processes, but also to biological testing on numerous animal models. A list of the most promising manufacturers of β-glucan is provided in Table 1.

β-Glucans will never be magic bullets able to nourish the body so that it can cure all diseases. Carefully planned experiments on animals and humans will result in highly active preparations able to properly support and strengthen the immune system.

Table 1: List of the most promising manufacturers of beta glucan

Company	Commercial Name	Source
Biopolymer Engineering	Beta Right	Yeast
Life Sources	Beta Right	Yeast
Norwegian	Beta Glucan	Yeast
MacroForce	Beta Glucan	Yeast
Goemar	Phycarine	Algae

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