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 extracellularly 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 Schizophyllum 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

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 mannann, 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

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us prevents its spread. Similarly, a β-glucan-binding protein is an important factor in boosting the immune system of crustaceans such as lobsters and shrimps. This protein enhances the activity of the prophenoloxidase-activating enzyme, which then induces the activation of phenoloxidase. In addition, glucan also directly activates the rmp hemocytes. Subsequent studies demonstrated that glucan has strong immunostimulating activity in other species, including earthworms, fish, chicken, mice, ticks, rabbits, guinea pigs, sheep, pigs, and cattle. Based on these results, we can conclude that β-glucan immunostimulation is active over a broad spectrum of biological species and is one of the first immunostimulants across the evolutionary spectrum. β-glucan is a biologically active polysaccharide considered to be an evolutionary extremely old stimulant of defense reactions of all nds. Clearly, the recognition of β-glucans as major comments of yeast, bacterial, and fungal cell walls belongs to the first defense mechanisms that evolved during phylogenetic processes.

The number of individual glucans is almost as great as the number of sources used for its isolation. Despite numerous studies performed all over the world, it is impossible to try that only one particular glucan is the optimal immunomodulator. Different physicochemical parameters, such as solubility, primary structure, molecular weight, branching, and polymer charge also play a role in determining whether the polysaccharide modulates immune reactions. From Yadome's recent review on the structural properties of glucans affecting biological activities, one may draw some conclusion. Branched or linear 1,4-β-glucans have very limited, if any, activity. Glucans with a 1,6 configuration 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 immunostimulating activity.

The pharmacokinetic parameters of β-glucan are extremely important for calculating therapeutic dose levels. Although larger glucans appear to be better because of their resistance to glomerular filtration and saturation of liver clearance, glucans that are too large have a higher potential for producing undesirable side effects. This condition is especially important when low molecular soluble glucans are used, as these glucans bind to individual CR3 molecules and prime these receptors to react only when later cross-linked by iC3b-coated prey. Here we can talk about a highly specific stimulation of one particular mechanism of the whole cascade of immune reactions.

In contrast to the water soluble low molecular weight glucans, β-glucans that are either large enough or insoluble can activate immune reactions nonspecifically, in absence of a specific target moiety. Various large glucans such as SSG, schizophyllan, or lentinan trigger a respiratory burst and stimulate the production of various immunostimulative substances such as IL-6, IFN, and TNF. In addition to their effective use via intraperitoneal and intravenous injection, glucans remain active even when given orally.

Despite all the research, cellular and molecular mechanisms responsible for glucan effects on the immune system are not completely understood. 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 nonspecific 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. The binding of β-glucans to the specific β-glucan receptor on macrophages and neutrophils is well documented. The binding results in Ca2+ influx through receptor-operated channels, regulated by protein kinase C. Anti-infective properties of yeast-derived β-glucans are summarized in a comprehensive review by Bleicher and Mackin. 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 considered to be the most important receptor mediating the clearance of iC3b-opsonized immune complexes by the phagocytic system. In addition to being a receptor for cytokotoxicity 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 binding, the CR3 is primed for cytokotoxic degradation in response to the binding of an iC3b fragment to a different part of the CR3 molecule. These data were further validated by the use of cells from CR3-deficient mice, which were resistant to the β-glucan effects.

**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. Glucan therapy strongly decreased septic morbidity. A multicenter, double-blind study found an optimal dosage of glucan in high-risk surgical 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. 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, CD8+ (NK cells), and LeuM3 (macrophages) cells among tumor-infiltrating lymphocytes increased.45 In a different study, the lifespan prolongation effects of lentinan in combination with chemotherapeutic agents had been confirmed in 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, and in Phase I/II clinical trials, but for unknown reasons, the trial 30-centre, 1,236 patients Phase III trials failed. In addition to immunomodulatory effects, β-glucans were also shown to reduce the total and LDL cholesterol levels of hypercholesterolemic patients.49

TOXICOLOGY OF GLUCAN

Despite numerous studies on the biological effects of lican, complete toxicology is still not fully established. The acute and subacute toxicities of lentinan were examined by intravenous injection into mice, rats, dogs, and monkeys. In all experiments, the LD₅₀ was more than 100 mg/kg. Using lower doses, no chronic toxicity was observed. In addition, no hypersensitivity was found in anaphylaxis tests.11 A 52-week oral toxicity study of a Candida licanus-derived β-glucan revealed no toxicity and no side effects up to the highest tested dose of 200 mg/kg/day. The lean-fed rats showed no physiological differences from control groups.19

SUMMARY

Investigations of substances nonspecifically affecting immune reactions are becoming more and more important from the view of potential clinical practice and from the view of veterinary medicine. More and more commercially important animals are farmed under stressful conditions, and the ability to boost their immunity by easily prepared commercial immunostimulants will strongly decrease morality induced by stress-related illnesses. In animals, glucans are being tested not only as nonspecific stimulants, but also as an addition to currently used vaccinations.20 In addition to veterinary and commercial practices, immunomodulatory polysaccharides are currently the focus of intensive clinical and clinical investigations on several parts of the body. The question “which glucan should be picked as the ideal immunomodulator?” remains open. However, one conclusion can be made — the yeast-derived glucans have the advantage of being a cheaper and a more defined source as well as having higher biological activity. Unfortunately, most commercial sources of β-glucans offer inferior products, derived either from crude extracts or primarily from coccid 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

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<tr>
<th>Company</th>
<th>Commercial Name</th>
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<td>Biopolymer Engineering</td>
<td>Beta Right</td>
<td>Yeast</td>
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<td>Life Sources</td>
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