This document provides an overview of current knowledge of the mode of action and clinical effects of immune modulating beta-1,3/1,6-glucans. Its intent is to provide a foundation for healthcare professionals to perform an independent assessment of the documentation concerning the use of beta-1,3/1,6-glucans as support for infection defense and modulation of immune responses in the body.
The primary function of the immune system is to protect the body against infections by pathogenic viruses, bacteria, fungi and parasites. It also plays a key role in removing dead body cells and repairing damage caused by strong light, irradiation and environmental toxins. Occasionally, the immune system may over-react or be brought out of balance. Such imbalances can result in immune disorders like rheumatoid arthritis, certain types of asthma, and other fairly common health conditions. The immune response can also be suppressed, resulting in reduced overall resistance to infections and impaired ability to counter development of cancer. Thus, a properly functioning immune system is a prerequisite for good health.

Beta-1,3/1,6-glucans are naturally occurring polysaccharides found in the cell walls of fungi and yeast but alien to the animal kingdom. Throughout evolution, the immune system has "learned" to recognize its molecular structure as a reliable warning of infection. In purified form, beta-1,3/1,6-glucan functions as a signal that alerts the immune system and prepares it to respond quickly and adequately to infections. However, beta-1,3/1,6-glucan is more than a potent immune-stimulant that renders animals and humans more resistant to any infectious organism. Beta-1,3/1,6-glucans have remarkable effects on a range of other conditions. This non-toxic, non-allergenic and non-immunogenic molecule enhances wound healing and repairs damage caused by strong light or irradiation. It also supports anti-cancer mechanisms in the body and has a beneficial effect on the overall health condition.

The multitude of effects of beta-1,3/1,6-glucan can be explained with reference to its very basic mode of action on the immune system. Beta-1,3/1,6-glucans interact with specific receptors on macrophages, dendritic cells, natural killer cells and granulocytes. These white blood cells are present everywhere in the body. In mucous surfaces and underlying mesenchyme tissues, they constitute a powerful defensive force that arrests and destroys infectious microbes at their port of entry into the body.

The interaction between beta-1,3/1,6-glucan and its specific receptors on immune cells in epithelia and superficial connective tissues corresponds to an initial event in a natural infection process and places these cells on highest alert to counter any subsequent infection.

White blood cells with beta-1,3/1,6-glucan receptors constitute the backbone of the body's innate immune system, which is the first line of defense against most infections. The same cells make up the mastermind of the entire immune system; they control and adjust the specific immune system response to infections, vaccines and allergens. The use of a beta-1,3/1,6-glucan to modulate innate immune mechanisms has therefore become a promising strategy for controlling and counteracting immune related disorders such as asthma, allergy, arthritis, and harmful inflammations. Innate immune mechanisms are important also for the body's own ability to eliminate tumor cells. Immune therapy has recently become an attractive and realistic anti-cancer strategy.

Frequently recurring infections and colds, allergy and asthma, arthritis pain, and chronic fatigue are conditions that are related in some way to weakened immunity or inadequate immune reactions. There are many possible causes of immune deficiencies; physical or mental stress, grief, sudden change in personal life, overwork, poor diet, aging, exposure to pollutants or irradiation, and possibly insufficient exposure to microbial products that provide natural exercise for the innate immune system.

Beta-1,3/1,6-glucans are immune modulating natural products that show the greatest promise as supplements that, by acting on basic mechanisms of immunity, will help to counteract the effects of weakened immunity.
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1. A functional description of immunity

1.1 Innate immunity: The "standing army" of infection defense

When professionals in medicine and immunology discuss immunity and immune mechanisms, they usually think of lymphocytes, antibodies and the infection defenses that develop some weeks after an infection or following vaccination. But these specific or adaptive immune defenses account for only a small part of the body's defense system. In biology as a whole, they are exceptions. Specific immunity is found in only about 1.5 % of all animal species.

As its name indicates, innate immunity is inborn. It provides an all-purpose defense against all infectious organisms. In biology as a whole, innate immunity is by far the most important infection defense system. It is, of course, difficult to quantify this statement, but the fact is that most first-time infections are arrested within hours or a few days, long before antibodies or antigen specific killer cells have been induced, before the specific defense system has had time to come to the rescue.

The importance of innate immunity is most clearly manifested by the fact that invertebrates like snails, shellfish and insects are without lymphocytes (and are therefore unable to be vaccinated in the same way as more elevated animals) but have an equal or better defense against infection than humans. Throughout evolution, we have maintained, in principle, the same innate infection defense system as the invertebrates depend on entirely. But in addition to it, we have also developed specific immunity for added security.- a defense system that is mobilized only when an infection overwhelms the innate defenses.

Our infection defense system falls into three distinct lines. The first one is formed by the epithelial, the second lies in the tissue structure (the so-called mesenchymal defense) and the third is the specific immune system connected with the function of lymphocytes.

There is a close functional connection between the innate immunity and the adaptive infection defenses. Innate immune cells in the tissue surfaces, including macrophages and dendritic cells, detect foreign elements and destroy invading microorganisms. Immune cells that become activated as a result of such natural challenge by infectious microbes produce signal molecules (cytokines) that recruit more immune cells to the infected region and prepare cells in the specific arm of the immune system (B- and T-cells) to produce antibodies against the intruder. In turn, when the specific immune system is provoked, cytokines are produced which have a positive feedback effect on innate immunity, making it even more effective.

1.2 Epithelial surface defense

The majority of immune cells (75 %) are found in body surfaces, particularly in gut endothelia, the largest immunological organ in the body. The skin area of an adult human is approximately 2 m² whereas the mucosal surface area is about 400 m². Almost all microbes resulting in illness invade one of the body's mucosal surfaces and most microbes are dealt with there before they can advance further. As a logical consequence, it is a good strategy in infection control to find ways to "Man the barrier" ( Nagler-Anderson 2001) without using the injection syringe.

Epithelial defense consists first of a dense mechanical barrier. Some epithelial cells have cilia that sweep the microbes toward the body openings. Next, the epithelial cells produce mucous containing carbohydrates that prevent microbes from adhering to the surfaces. Most importantly, however, the cells produce peptide antibiotics, iron-binding substances and lysozyme.

Peptide antibiotics (defensins) have a wide-ranging effect with an efficacy per mol similar to tetracyclines (Hancock and Scott 2000; Ganz and Lehrer 2001). There is no proven acquired resistance against natural defenses, something that can be due to their crude mode of action-dissolving the cell membranes of microbes. Production of defensins and other peptide antibiotics is mostly constitutive. They are produced continuously without stimulation of the cells that produce them. However, it is known that the concentration of defensins increases under infection (Ganz and Lehrer 2001), which must mean that the production of these important anti-microbial principles in tissue surfaces can be induced to some extent.

One obvious important task for medical research in the future will be to find well-defined and safe substances which, when applied to the mucous surfaces, can stimulate increased production of defensins. The same applies to lysozym and to iron-binding substances (e.g. lactoferrin) which compete with infectious microbes for iron because it is vital for their propagation. Since all of these anti-microbial principles in mucous are produced by innate immune cells in epithelial surfaces, immune-stimulants that target these cells are the most likely candidates.

1.3 The mesenchymal defense

The term mesenchyme is used for connective tissues, veins, and blood cells, all of which are coordinated and function together. Serious genetic innate errors in the mesenchymal system do not arise, undoubtedly because an individual with such errors is not viable even in pregnancy and would be aborted.

The mesenchymal infection defense is first and foremost connected with tissue macrophage function. Tissue macrophages are found everywhere in the body where there is connective tissue and veins including the brain where they are known as microglia.

Tissue macrophages are phagocytes. They ingest microbes and dissolve them. If a small quantity of microbes enters the connective tissue, they are quickly and completely eliminated by macrophages through phagocytosis. In addition, if several microbes have invaded, the macrophages will react by producing cytokines, especially interleukin 1 (IL1) and tumor necrosis factor (TNF). These cytokines induce changes in the endothelial tissues and start an acute inflammation. Consequently, granulocytes with an enormous capacity for phagocytosis and bacterial killing are summoned.

Local acute inflammation on a small scale should be accepted as a normal process. From time to time, all humans have a minor inflammation in some area of the body. The concentration of the acute phase C-reactive protein (CRP) in blood reflects the degree of inflammation and may therefore be a sensitive marker of infection and other disease conditions including coronary artery disease (Libby and Ridker 1999).

1.4 Innate immune cells monitor the microbial environment

The most well-known and well-described phenomenon in immunology is the production of specific antibodies and induction of antigen-specific T-cells following infection
or vaccination. But the innate immune system response to infections has not been understood until recent years. As a result of a paradigm shift in immunology during the last 10 years, innate immune mechanisms have become a predominant area of immunology research. From a subordinate status in immunology, innate immunity has received "new respect" (Gura 2001) in recent years and has obtained the position as the "mastermind" of immunity.

Innate immunity not only constitutes the frontline defense, it also controls and coordinates how the specific part of the immune system reacts to various infectious challenges and to environmental factors such as allergens. Some excellent review papers can be recommended for an update on the status of innate immunity in modern immunology: Gura 2001; Levy 2001; Basset et al. 2003; Beyan et al. 2003; Lolis and Bacala 2003; Sartor 2003; Sfondrini et al. 2003; Wakimoto et al. 2003.

The innate immune cells like macrophages, dendritic cells, granulocytes and natural killer cells are equipped with surface receptors that discriminate between different microbial substances. To this date, a total of ten so-called "Toll-like receptors" (TLR) have been found, numbered TLR 1 to TLR 10. For instance, bacterial lipopolysaccharides (LPS) interact with TLR 4, bacterial DNA with TLR 9 and beta-1,3/1,6-glucans with TLR 2 and/or 6. Although all these microbial products activate innate immune mechanisms, the signals produced by the immune cells are different as are the biological responses. LPS elicits TNF production and other cytokines which induce inflammation and fever, whereas beta-1,3/1,6-glucans induce a strong Interferon-gamma production.

Innate immunity is no longer regarded as an unspecific and "blind" defense mechanism. We now know that innate immune cells are equipped with sophisticated antennas which continuously monitor the nature of the microbial environment. Our immune system has obviously evolved in a natural environment with hostile micro-organisms and has probably become dependant on their continuous challenge. This information will aid us in learning about the innate immune system's response to the microbial world and to microbial alarm molecules and can eventually help us to manipulate immunity to generate the most appropriate response. Herein lies the hope of finding the most appropriate methods to prevent inappropriate immune responses such as those causing asthma and allergy. Modern thinking leans toward stimulating immunity with substances that skew the immune response in so-called Th-1 direction rather than suppressing symptoms of such inadequate immune reactions (illustrated simplified in Figures 3 through 6).
1.5 The specific immune system

The body's third defense line is the specific immune system. Connected with the lymphocyte system, it is characterized by extreme specificity and memory. Vaccination gives especially effective protection against a specific microbe but against no others. The memory, that is to say the duration of the effect, can last for years or even lifelong.

Since the activity of the specific immune system has such a significant effect and the studies of this system have produced so many valuable impulses even to fields of research other than immunology, it is easy to overlook that the non-specific infection defense connected with epithelial and mesenchymal tissue has considerably greater significance than the specific immune system, both generally within biology and also in our daily fight against infection.

The mesenchymal and lymphocyte system are cooperative with one another. Cells in the mesenchymal system often have to present antigens to lymphocytes in order to achieve an effective immunization. In the future development of vaccines, it seems a sensible strategy to try to imitate the natural infection process by activating the innate immune system in epithelial and mesenchymal tissues with the help of nature's own "danger signals" (pathogen associated molecule patterns), targeting the TLRs. By that method, it might be possible to achieve a more natural and effective adjuvant effect than with today's vaccine injections.

2. Activation of innate immunity by microbial products

2.1 Immune-stimulants and immune-modulators

Macrophages can either be resting - which is normal - or be active. Activation can occur as a result of a normal infection process or by isolated bacterial and fungal products such as immune-stimulants or immune-modulators. The response to infection, or to microbial products, is an example of immune mechanisms developed through evolution that quickly register structures unique to individual microbes and which therefore have become reliable alarm signals for infection. Examples of bacterial agents which can activate macrophages directly are lipopolysaccharides, peptidoglycans, lipothionic acid, lipoarabinomannan, lipopeptides, and beta-1,3-glucans (Raa 1996). Examples of stimulating components in fungi or protozoa are beta-1,3-glucans, mannan and glycosyl phoshatidylinositol. There are also substances which inhibit the reaction of macrophages, e.g. lipophosphoglycan from "Leishmania donovani" (Aderem and Ulevitch 2001).

The natural introduction to the body of microbial macrophage activating substances occurs in the form of infections by bacteria, viruses, fungi or protozoa, and with the natural and unavoidable intake of microbial products from the milieu we inhabit. It is possible that, through evolution, the body has become dependent on a certain intake of microbial products in order to function adequately, or "Give us this day our daily germ" (Rook and Stanford 1998).

For many years, researchers have tried to find substances that can induce the same responses as those occurring during early events in a normal infection process. The idea has been to develop controllable procedures that alert innate defenses to respond quickly and effectively to infections without causing serious inflammation or negative side-effects associated with an infectious disease. This is a promising strategy of disease prophylaxis and in curative treatment of certain immune related diseases. The most promising product candidates are bacterial and fungal components of beta-1,3-glucan nature and fragments of bacterial DNA (Medzhitov 2001).

Beta-1,3/1,6-glucans have a long history in medical practice and are therefore closer to far-reaching pharmaceutical applications. Beta-1,3/1,6-glucans have a very basic mode of action and may affect many immune related disorders. Further, existing and historical use of beta-1,3/1,6-glucans have already given a strong foundation of anecdotal information about the most promising indications for further clinical development.

2.2 Immune modulating beta-1,3/1,6-glucans and medical indications

In experimental systems, Beta-1,3/1,6-glucans have proven extremely effective as immune stimulants that enhance resistance to infection by viruses, bacteria, fungi and parasites (Seljelid et al. 1987; Kaneko and Chihara 1992; Maeda et al. 1994; Williams et al. 1998). They show no toxic effects, even in concentrations much higher than those normally used in fighting infection. Beta-1,3/1,6-glucans work not only by increasing the ability of the organism to kill microbes, but they also protect against shock produced by bacterial endotoxins (lipopolysaccharides). This protection is connected with the fact that beta-1,3/1,6-glucan counteracts endotoxin-induced production of tumor necrosis factor (Seljelid et al. 1997) and lipopolysaccharide-induced toxicity (Williams et al. 1995; Vereschagin et al. 1998; Rylander and Holt, 1998; Soltys and Quinn, 1999).

The mesenchymal system is also important in relation to the healing of wounds. Macrophages produce growth factors and thereby regulate healing of wounds. A process that can be accelerated by beta-1,3/1,6-glucan (Portera et al. 1997).

Experimental studies have shown striking anti-tumor effects of macrophage-stimulating beta-1,3/1,6-glucans (Seljelid 1989; Sveinbjørnsson et al. 1998). Beta-1,3/1,6-glucan is used to counteract immune-suppression resulting from radium treatment and chemotherapy and to stimulate the body's own fight to expel cancer cells (Jong and Birmingham 1993). The majority of these studies have been carried out by injecting beta-1,3/1,6-glucan but also oral administration produces a notable systemic effect. This result was unexpected since beta-glucans are not absorbed from the gut into the blood. It was therefore a noteworthy observation when researchers at Sloan Kettering Institute of Cancer Research (New York) showed that orally administered beta-glucans enhance the anti-tumor effects of injected monoclonal antibodies against various cancer types (Cheung et al. 2002). However, the observation is in line with experiments from Japan and experience with farm animals receiving beta-1,3/1,6-glucan as a dis-
ease preventing feed additive. The fact that beta-1,3/1,6-glucans act via the oral route to potentiate injected monoclonal tumor antibodies has indeed opened up a complete new strategy in cancer therapy. The observation adds nicely to the experience from Oriental medicine where oral beta-glucans from mushroom have been used for many years as stand-alone anti-cancer medicines. It is also a nice confirmation of the results from studies with beta-1,3/1,6-glucans in feed or drinking water for farm animals.

2.3. Use of beta-1,3/1,6-glucans in domestic animals

For more than ten years beta-1,3/1,6-glucan has been used with great success to prevent diseases in aquaculture and traditional domestic animal husbandry, mostly as a feed-additive, but also as an adjuvant in vaccines and for immersion of small fish and cultivated shrimp. The results are widely accepted after worldwide use with many different animal species, both aquatic and terrestrial. Beta-1,3/1,6-glucan contributes to increased disease resistance (reduced mortality) and, possibly as a result of this, to better weight and food uptake (Robertson et al. 1990).

Beta-1,3/1,6-glucan is used in feed for piglets and calves to reduce infections during weaning, for chickens and broilers against virus and bacterial infections, and for dogs against various immune related disorders. In piglets beta-1,3/1,6-glucan causes reduced level of haptoglobin in the blood (Dritz et al. 1995), a parallel to the CRP-lowering effect of beta-1,3/1,6-glucan as oral supplement to humans, shown in recent a "double blind" study with individuals with mild or moderate hypercholesterolemia (in preparation).

The use of beta-1,3/1,6-glucan in domestic animal husbandry is based on a comprehensive scientific documentation and practical experience which is very relevant also for human medicine. It not only constitutes a reliable safety and efficacy test for beta-1,3/1,6-glucans, but has provided "proof-of-concept" for eventual human use. For instance, animal studies have shown that beta-1,3/1,6-glucan administered orally or as a nasal spray, has a systemic effect on immunity and may act as an adjuvant for both injected and mucosal vaccine antigens (Raa et al. 2002). This is in accordance with the observations by Cheung et al. (2002) that orally administered beta-glucan enhances the anti-tumor effect of monoclonal antibodies that are injected.

It appears that beta-1,3-glucan in high molecular weight or micro-particle form is not taken up from the intestine in noticeable amounts and transported around in the blood. Nevertheless the substance produces a systemic effect on animals. This can mean that the substance reacts with glucan-receptors on cellular extensions from macrophages and the dendritic cells in the underlying lymphoid tissue (Nagler-Anderson 2001).

3. The history of beta-1,3/1,6-glucans

3.1. Beta-1,3/1,6-glucans in Oriental medicine

Extracts of the Shiitake mushroom (Lentinus edodes) in Japan and of the mushroom known as "Lingzhi" (Ganoderma lucidum) in China have a recognized position in traditional medicine of the Orient. The oldest Chinese medical dictionary ("Shen Lungs Medica") and the "bible" of Chinese herbal medicine ("The Chinese Herbal Materia Medica") rate Lingzhi extracts as a "spiritual essence" which strengthens the body and cures cancer, urinary disorders, fever diseases and arthritis/rheumatism. The wisdom of ancient medicine, generated by experience, has gained strong support from Western scientific research which has indeed confirmed that beta-glucans extracted from these mushrooms inhibit tumor growth and increase resistance to infections by virus, bacteria and parasites.

3.2. Zymosan and beta-1,3/1,6-glucan in baker's yeast

More than 50 years ago it was discovered by scientists in the United States (Pillimer and Ecker) that disrupted and enzyme digested baker yeast contained a material which interacted with serum components that are involved in the destruction of infectious microorganisms. In 1957, Benaceraff and Sebastian presented a paper on the stimulating effect of a crude cell wall preparation from baker's yeast (called zymosan) on macrophages. In the 1960s, DiLuzio and his coworkers in New Orleans showed that the active component in zymosan was beta-1,3/1,6-glucan and that this component had the ability to enhance disease resistance and limit growth of tumors in humans.

During the last 20-30 years, much work has been devoted to develop methods to extract this beta-1,3/1,6-glucan from baker's yeast in pure and active form, and to reveal its chemical structure and mode of action on the immune system. Today the mode of action of the yeast beta-1,3/1,6-glucan is known in great detail. Its ability to prevent infections caused by virus, bacteria, fungi and parasites has been documented in hundreds of scientific papers, and confirmed by practical experience.

The few toxicological events observed after administration of yeast beta-1,3/1,6-glucan are restricted to intravenous or intraperitoneal injection (Di Luzio et al. 1980; Takahashi et al. 2001; Williams et al. 1996).

4. Chemistry, properties and manufacture of glucans and beta-1,3/1,6-glucans

4.1. What is a glucan and what determines its immune-stimulating property?

All macro-molecules containing glucose as the only building block are called glucans (macro-molecules containing other sugars than glucose are called glycans). Starch and cellulose are well-known examples of glucans, where the glucose molecules are linked together in so-called alpha- and beta-1,4-linkages, respectively. Such glucans have no effect on the immune system.

The common feature of glucans which have the ability to activate the immune system is a chain of glucose molecules linked together in so-called beta-1,3-linkages. However, to be active there must also be "branches" of
glucose molecules attached (by beta-1,6-linkage) to this beta-1,3-glucan chain. For example, the sea-weed beta-1,3-glucan laminarin, which is almost depleted of such branches, is not active as an immune stimulant. On the other hand, branched beta-glucons (called beta-1,3/1,6-glucons according to chemical nomenclature rules) are very potent, non-toxic immune-stimulants.

The beta-1,3/1,6-glucons scleroglucan, lentinan and schizophyllan which are extracted from medicinal mushrooms are active immune-stimulants. Their efficacy is lower, however, than that of a fully branched beta-1,3/1,6-glucon extracted from baker's yeast because the side branches of the mushroom products consist of only one glucose whereas the side branches of the beta-1,3/1,6-glucon from yeast consists of chains of glucose molecules (Engstad and Robertsen, 1993; 1994 and 1995; Bohn and BeMiller 1995; Suzuki et al. 1998). To match perfectly into the receptor on the white blood cells, the length of the branches should be at least 2 glucose molecules (Engstad and Robertsen 1995).

The beta-1,3/1,6-glucon present as a structural component inside the cell walls of baker's yeast has a molecular structure that fits with the glucan receptor on the white blood cells. Baker's yeast is therefore a suitable raw material for extraction of a beta-1,3/1,6-glucon with high biological efficacy. However, it is difficult to release this beta-1,3/1,6-glucon molecule from the cell wall structure. The challenge has been to remove those molecules (e.g. manno-proteins) which, in the intact cell wall, are attached to the end-points of the side-branches without causing de-branching of the beta-1,3-glucan chain and, as a consequence, loss of efficacy.

The high efficacy of yeast beta-1,3/1,6-glucon compared to glucons and glycans from other sources as an immune stimulant was shown by Seljelid et al. in 1981.

4.2. Brewer's yeast
Brewer's yeast is not a suitable source of bio-active beta-glucan for two reasons. First, the total content of beta-1,3-glucan is very low compared to baker's yeast and second, the beta-1,3-glucan present has a low number of side-branches.

4.3. Oat and barley glucons
The beta-glucons present in oat and barley have a different composition than those present in yeast and mushrooms. In the beta-glucons of oat and barley, the glucose molecules are joined partly by beta-1,3- and partly by beta-1,4-linkages. These are soluble polymers which lower serum cholesterol and glucose levels when used in the human diet at high inclusion levels (15 grams per day) (Davidson et al. 1991). However, these effects are thought to be due to a "fiber process" whereby cholesterol and bile salts are eliminated from the ileum along with the glucan fiber. The effect is probably not mediated via the immune system.

4.4. Micro-particulate beta-1,3/1,6-glucon from baker's yeast
The conventional procedure for extraction and purification of the beta-1,3/1,6-glucon from baker's yeast consists of removal of cytoplasmic proteins by alkaline treatment followed by treatment in acid to remove the minerals, lipids and manno-proteins. The manno-proteins cover the surface of the intact yeast cell and they are covalently bound to the beta-1,3/1,6-glucon which constitutes a net-like structure in the inner part of the cell wall. The manno-proteins have to be removed for two reasons-first, to expose the beta-1,3/1,6-glucon structure (the active principle), and second because the manno-proteins are a potential allergenic constituent of yeast. Removal of manno-proteins is a critical step in the extraction process during manufacture of bioactive beta-1,3/1,6-glucon. It is quite probable that one may easily end up with a beta-1,3-glucan where the side branches have been stripped off and the immune stimulating activity is gone.

The result of the extraction of a beta-1,3/1,6-glucon with high biological activity (US Patent No. 5,401,707, EP No. 0466037) is small "bags" with a size in the range 1 to 3 microns. These micro-particles expose a great number of free glucose ends that can bind to receptors on white blood cells.

The high bioactivity of yeast beta-1,3/1,6-glucon compared to other beta-1,3/1,6-glucons (from mushroom) is one of the reasons why yeast beta-1,3/1,6-glucon attracts more and more attention as a pharmaceutical product candidate and as health supplement. Another reason is that the product comes from a so-called GRAS-organism (Generally Regarded As Safe): food grade baker's yeast produced under the regulations of good manufacturing practice.

4.5. Soluble beta-1,3/1,6-glucons from baker's yeast
To make a bioactive soluble beta-1,3/1,6-glucon, one has to start with a pure and bioactive micro-particulate product. There are several ways to do this. The most straightforward is to react the beta-1,3/1,6-glucon micro-particles with CI-acetic acid, a process called carboxy-methylation, which introduces negatively charged acetate groups at random in the beta-1,3/1,6-glucon molecule. The product, CM-glucan, has been used in cosmetic products. But the disadvantage is that the beta-1,3/1,6-glucon looses much (sometimes all) of its bioactivity when end-positioned glucose molecules (which bind to glucan receptors) are carboxy-methylated. Another solubilization procedure consists of reacting the micro-particulate beta-1,3/1,6-glucon with phosphoric or sulfuric acid to produce phosphorylated or sulphated beta-1,3/1,6-glucons that become water soluble as a result of introduction of negatively charged groups on the molecule. But also these soluble products have the disadvantage of lost bioactivity, due to altered structure of the end-positioned glucose molecules.

Much work has been invested to find ways to solubilize the yeast beta-1,3/1,6-glucon in native form, without chemical modifications as described above. One procedure is described in Norwegian patent (300,692) and EPO-patent (EPO759089), where the resulting product is a 100 % pure native yeast beta-1,3/1,6-glucon with very high biological activity and excellent safety profile (see 6.4).

5. Mode of action and biological effects
5.1. Recognition of beta-1,3/1,6-glucons by phagocytic cells
Since the first studies showing that the zymosan was able to activate a population of white blood cells, it has been postulated that specific receptor(s) on the cells, recognizing the beta-1,3/1,6-glucon structures, was/were facilitating the action of zymosan. During recent years the term Pathogen Associated Molecular Pattern (PAMP) Receptors have been used to describe receptors on cells of the innate immune system, recognizing conserved microbial structures like beta-1,3/1,6-glucons.
Several different receptors on phagocytic cells that recognize betas-glucans have been identified: the CR3 (CD11b/CD18) (Xia et al. 1999), the dimers of the Toll like receptors 2 and 6 (Ozinsky et al. 2000), and the Dectin-1 (Brown & Gordon 2001). The exact specificity of the different receptors have still not been fully elucidated, but there are studies showing that the non-reducing terminal end of beta-1,3-linked glucan chain is likely to be a main target for recognition by the receptors (Engstad & Robertsen 1994).

It has been demonstrated that the ability to induce an immune response is largely dependent upon the ability of beta-glucans to cross-link several receptors, implicating that beta-glucan preparations that present multiple receptor binding epitopes would be the most potent immunostimulants (Poutsiaka et al. 1993; Goldman 1995).

In addition to the beta-glucan recognition receptors, it has been shown that particulate beta-glucans interact with serum opsonins (Konopski et al. 1991). Interactions with the beta-glucan receptors on cells of the innate immune system initiate the reactions by which beta-1,3/1,6-glucans modulate immunity. Such receptors exist also on epithelial cells of the alimentary tract where interaction with beta-glucans may cause signal transduction to underlying immune cells (Nagler-Anderson 2001). This explains why beta-1,3/1,6-glucans are also active when administered orally. Beta-glucans of different origins and different structure are all believed to act through the same route, i.e. through an initial interaction with beta-glucan receptors. Their efficacy as immune-modulators, however, is determined by differences in receptor affinity towards the different beta-glucan preparations and also their ability to cross-link receptors (Williams 1997; Müller et al. 2000).

5.2. Induction of IFN-gamma in gut lymphocytes by micro-particulate beta-1,3/1,6-glucan

Oral administration of micro-particulate yeast beta-1,3/1,6-glucan to mice caused a marked increase in number of intraepithelial lymphocytes in the gut, in particular of the gamma-delta T-cell population. In addition, the endothelial lymphocytes in the gut switched on high IFN-gamma production (Tsukada et al. 2003). Micro-particulate yeast beta-1,3/1,6-glucan has a direct effect on innate immunity, and, in particular, it enhances cellular immunity, strengthening resistance against viral infections. It also shows that the beta-1,3/1,6-glucan is possibly skewing the immune response in Th-1 direction. If so, it is in accordance with observations that beta-1,3/1,6-glucans counteract allergy and asthma despite their stimulating effects on immunity.

5.3. Effect on mucous: Induction of antigen specific T-cells in spleen

Both micro-particulate and soluble native beta-1,3/1,6-glucan from yeast have systemic effects on the immune system when administered onto mucosal surfaces, either in the nasal cavity or in the gut. A striking illustration is the effects (see Figure 9) of the micro-particulate beta-1,3/1,6-glucan preparation when administrated together with influenza vaccine antigens into the nasal cavity of mice (Raa et al. 2002). While the influenza virus antigen alone caused a moderate "priming" of antigen specific T-cells in the spleen, co-administration with the micro-particulate beta-1,3/1,6-glucan (occurs also with the soluble preparation) caused up to a 30-fold increase (depending on dosage) in the ability of spleen T-cells to respond to later exposure to the same antigen. This response was not correlated with the IgG-antibody response to the influenza virus antigen, indicating an effect primarily directed towards enhanced cellular immunity. Such studies have shown that beta-1,3/1,6-glucan can also modulate and down-regulate certain immune responses.

5.4. Higher milk IgG

To protect suckling piglets from bacterial gut infections, sows are vaccinated by injections of antigens from such bacteria in order to get a highest possible antibody level in the milk. It has been demonstrated that beta-1,3/1,6-glucan from yeast, given in the sow diet, results in a higher specific IgG secretion in the milk compared to the vaccination alone (Decuipere et al. 1998).

6. Safety

6.1. General

Fungal beta-1,3/1,6-glucans have a 3000-year long history as a medical treatise in traditional Eastern medicine, a practice also adapted in clinical cancer therapy in Japan today (Noda et al. 1992). The research on beta-1,3/1,6-glucans in the Western Hemisphere has mainly focused on beta-1,3/1,6-glucans from baker's yeast (Saccharomyces cerevisiae). The compound is regarded as very safe in use when administered orally or topically. It is isolated from, and a major part of, a GRAS organism and it is composed solely of glucose units. For years, beta-1,3/1,6-glucans isolated from yeast have been used as dietary supplements for humans (Bell et al. 1999) and for topical applications (Zülli et al. 1997) with no reports of adverse or toxic effects. The few
toxicological events observed after administration of yeast beta-1,3/1,6-glucans are restricted to intravenous or intraperitoneal injection of beta-1,3/1,6-glucans (Di Luzio et al. 1980; Takahashi et al. 2001; Williams et al. 1996).

6.2. Experience from practical use

More than 30 different scientific papers investigating primarily the efficacy, but to a certain extent also the safety, of yeast beta-1,3/1,6-glucan have been published in peer reviewed journals. These papers describe several hundreds of experiments where the yeast beta-1,3/1,6-glucan had been administered to the animals by injection, in feed, by intubation, by immersion, in the water supply etc. So far no reports have indicated any toxic effect of the beta-1,3/1,6-glucan preparations. In addition to published papers, feed companies have carried out more than 100 feeding trials to establish the optimal feeding regimes for implementing the yeast beta-1,3/1,6-glucan in feed to animals like pigs, calves, broiler, dogs, cats, rabbits, several fish species, and larvae and juveniles of many aquatic species. In some of trials the beta-1,3/1,6-glucan were fed throughout the whole life cycle of the animal. In addition yeast beta-1,3/1,6-glucan has been used as a dietary supplement for several years. On this background it seems safe to assume that the yeast beta-1,3/1,6-glucan is not encumbered with any toxicological side effects.

6.3. No immunogenicity

In purified form beta-1,3/1,6-glucans are not immunogenic—no antibody can be raised against this molecular structure. Immunogenicity is very important for safety evaluations of pure products, in particular with regard to allergy. Antibodies that have been raised against fungal and yeast preparations of beta glucan are therefore always directed against other constituents in the preparations, such as mannose-proteins in yeast and other glycoproteins in mushrooms.

6.4. Formal safety studies: Basis for clinical trials

6.4.1. Micro-particle yeast beta-1,3/1,6-glucan

A parallel, double blind, placebo controlled study with 140 individuals with mild to moderate hypercholesterolemia, but otherwise healthy, showed no adverse reactions or signs which indicate any safety concerns related to a daily intake for 8 weeks of 0.7 grams of a yeast beta-1,3/1,6-glucan preparation (Ref. Biotec Pharmacon ASA, N-9008, Tromsø/Oslo, Norway). The same product has been used for years as supplement for humans, and no reports on adverse effects have been reported.

6.4.2. Soluble native beta-1,3/1,6-glucan from yeast

The following independent toxicity tests (Covance Laboratories Ltd. England) of soluble native yeast beta-1,3/1,6-glucan have been completed: 1) "Reverse mutation in five histidine-requiring strains of Salmonella typhimurium" (52 pages); 2) Induction of chromosome aberrations in cultured Chinese hamster ovary (CHO) cells (46 pages); 3) Single dose intravenous toxicity study in the mouse - estimation of approximate lethal dose (22 pages); 4) Single dose intravenous toxicity study in the rat - estimation of approximate lethal dose (23 pages); 5) 28 day oral (Gavage) administration toxicity study in the rat (188 pages). The results showed no toxicity in any of these tests which were carried out according to Good Laboratory Practice. Even at intravenous dosages as high as 400 mg/kg in mice or rats, there was no mortality and no clinical signs of reaction to the product (Ref. Biotec Pharmacon ASA, N-9008, Tromso/Oslo, Norway).

Subsequently, a human safety test has been carried out at the National Hospital in Oslo with healthy volunteers. No toxicity or any side-effects was reported with healthy volunteers receiving 100, 200, and 400 mg/day orally for 4 consecutive days (Ref. Biotec Pharmacon ASA, N-9008, Tromso/Oslo, Norway). Based on complete and satisfactory safety documentation of this product, it is accepted for clinical trials in humans.

Prior to these safety studies, several clinical safety studies have been performed with the poly(1-6)-B-D-glucopyranosyl-(1-3)-B-D-glucopyranose (PGG-glucan) named Betafectin provided by Alpha-Beta Technology, Inc., Worcester, MA, USA. The investigational drug PGG-glucan was derived from a proprietary, non-recombinant yeast strain of Saccharomyces cerevisiae. Initial in vitro characterizations of the product had revealed a high affinity for receptors of human monocytes and neutrophils. Clinical trials were conducted with the PGG-glucan in healthy volunteers and the results showed that a single intravenous dose of 0.05 to 2.25 mg/kg was safe and well tolerated. Clinical assessments of physical conditions, vital signs and electrocardiograms showed no clinically significant abnormalities, and the product did not produce persistent fever, nausea, myalgia or bone pain. There were transient increases in total white blood cell counts, and in the monocyte and neutrophil counts, indicating a possible clinically useful response. Furthermore, at doses of 0.5 mg/kg and 2.25 mg/kg, an increased neutrophil and mono- cyte microbial killing activity against S. aureus, was observed.

A: A soluble beta-1,3/1,6-glucan has been subjected to pre-clinical safety evaluation in mice, rats, guinea pigs and rabbits (Williams et al.1998). ICR/HSD mice and Harlan Sprague-Dawley rats received a single i.v. injection of soluble glucan in doses ranging from 40 to 1000 mg/kg. Soluble glucan administration did not induce mortality, appearance or behavioural changes in mice or rats. In subsequent studies, mice and guinea pigs were injected i.p. with glucan (250 mg/kg) for 7 consecutive days. ICR/HSD mice gained weight at the same rate as the saline-treated controls. In contrast, guinea pigs receiving i.p. injections of soluble glucan showed a significant (P less than 0.05) 10-13% decrease in weight gain over the 7-day period. No other toxicological, behavioural or appearance changes were noted. To examine chronic toxicity, soluble glucan was administered twice weekly for a period of 30 or 60 days to ICR/HSD mice in the dose of 40, 200 or 1000 mg/kg. No deaths were observed in any group. Chronic glucan administration did not alter body weight, liver, lung or kidney weight. However, a significant splenomegaly was observed in both the 30 and 60-day study. Histopathologic examination showed no tissue alterations at 40 or 200 mg/kg. However, at 1000 mg/kg a mononuclear infiltrate was observed in the liver. Pyrogenicity testing, employing New Zealand white rabbits, revealed that parenteral glucan administration (5 mg/kg) did not significantly alter body temperature. These data indicate that the systemic administration of soluble glucan, over a wide dose range, does not induce mortality or significant toxicity, an important consideration in preparing soluble glucan for parenteral administration to human populations.

B: Lentinan (beta-1, 3-glucan) was studied on the acute toxicities using both sexes of mice (ICR) and rats (CD) treated intravenously (i.v.) intraperitoneally (i.p.), subcutaneously (s.c.) and orally (Moriyuki and Ichinuma 1980). LD50 values in mg/kg body weight were essentially the same regardless of species as well as sexes and estimated as follows: 250-500 (i.v.) greater than 2500 (i.p., s.c., and p.o.). Cyanosis, convulsion and death were observed in both species of animals administered (i.v.) with only higher dosages of lentinan. No remarkable toxic signs being specific from lentinan were observed in any cases of treatment, i.p., s.c., and p.o. Gross findings: enlargement of the spleen (i.v., i.p., s.c.) and coarse nodular surface of the kidney (i.v.) in the both species of animals, erythema of the ears (i.v., i.p., s.c.) in mice, mense teric petechial haemorrhage of the lung and abdomen (i.v.) enlargement of...
the mesenteric lymph nodes (i.v.) and oedema of the diaphragm and intestine (i.p.) in rats were observed. In parallel, another sample of lentinan for clinical use prepared by freeze-dried procedure was tested in both sexes of mice and rats treated by i.v. alone, comparing with an original sample mentioned above. So far as the acute toxicities of lentinans concerned, no significant differences between the two preparations were observed.

C: Mice, rats and rabbits of both sexes were used for pharmacological and acute toxicological study with antitumour polysaccharide, schizophyllan (Matsuo et al. 1982). The physiologically tolerable maximum doses SPG (schizophyllan) were administered by i.m., s.c., i.p. and i.v. routes, respectively. No dead case was found in any species and any administration route during 14 days after a single administration of up to 2000 mg/kg i.p., 300 mg/kg i.v.; 100 mg/kg i.m., and 2000 mg/kg s.c.

Sub-acute toxicity was evaluated after s.c. administration on alternate days for 3 months with doses up to 40 mg/kg. No dead cases were found, no significant alteration on body weight, food consumption of water intake was observed.

7. Selected pre-clinical "proof-of-concept" studies

7.1. Effects of postirradiation carboxymethylglucan administration in mice (Hofer et al. 1995).

The haemopoiesis-enhancing ability of a soluble glucan derivative, i.e. carboxymethylglucan (CMG), was investigated in gamma-irradiated mice. CMG was administered i.p. at (a) single dose of 6 mg 2 h postirradiation, (b) four 6 mg doses in the first 4 days postirradiation, (c) four 1.5 mg doses at the same time intervals. Indications of granulopoiesis and inflammatory side effects (liver weight increase and hepatic granulomas) were investigated in mice irradiated with a sublethal dose of 7 Gy. All three CMG-treated groups of mice were found to exhibit enhanced haemopoietic recovery in comparison with the controls. The mice repeatedly given the 6 mg CMG doses (group b) showed the most rapid recovery of all the evaluated parameters of granulopoiesis, but also the most pronounced hepatic side effects were found in these mice. When survival of mice was recorded in lethally (9 Gy) irradiated animals, the best protective response were obtained following the repeated administration of the 1.5 mg CMG dose, the survival by day 30 in this group being significantly higher not only in comparison with the controls but also with the mice repeatedly given the 6 mg dose of CMG. The results suggest that the postirradiation CMG administration can be useful for enhancing radiation-suppressed haemopoiesis. However, repeated larger CMG doses may produce side effects that compromise the overall survival of irradiated mice.


Alkaline extraction of insoluble S. glucanum exopolymers produced a soluble scleroglucan composed of a triple-helical beta-1,3-linked glucopyranose backbone with single beta-1,6-linked glucopyranosyl branches every third subunit. Scleroglucan has a weight average molecular mass of 1.56 x 10(6) Da, a weight average root mean square distance from the centre of gravity of the molecule to its farthest elements of 51.8 nm, a polydispersity (weight-average molecular mass/number average molecular mass) of 1.83 and intrinsic viscosity of 3.081 dl/g. Scleroglucan (250 mg/kg, intravenously) stimulated in vivo murine macrophage phagocytic activity (66%, P less than .001) and increased in vitro macrophage tumour cytotoxicity against syngeneic tumour targets by 124% (P less than .001). Scleroglucan enhanced (P less than .001) murine bone marrow proliferation in a biphasic manner by up to 328%.

Scleroglucan therapy increased survival of mice challenged with syngeneic lymphoma, melanoma or adenocarcinoma. AKR/J mice bearing syngeneic lymphoma (1 x 10(3) cells, intraperitoneally) demonstrated increased (P less than .001) long-term survival (100% vs. 0%, greater than 64 days). C57Bl/6J mice bearing syngeneic melanoma B16 (5 x 10(5) cells, subcutaneously) demonstrated increased long-term survival (64% vs. 0%, P less than .05). C57Bl/6J mice bearing syngeneic adenocarcinoma BW10232 (1 x 10(5) cells, subcutaneously) demonstrated increased (P less than .05) median survival time. In addition, scleroglucan prophylaxis increased resistance of mice to challenge with Staphylococcus aureus, Candida albicans and mouse hepatitis virus A-59. Scleroglucan did not induce toxicity or hepatomegaly. It was concluded that the water-soluble beta-1,3-linked scleroglucan stimulates immunity, modifies experimental neoplastic disease and increases resistance to microbial challenge.


This report describes the development, characterization and preclinical efficacy evaluation of water-soluble glucan sulphate. Glucan sulphate was derived from insoluble beta-1,3-D-glucan isolated from Saccharomyces cerevisiae. The proposed repeating unit empirical formula of glucan sulphate is [(C6H10O5)5.3H2SO4]n. Two polymer peaks were resolved by aqueous multi-angle laser light scattering (MALLS) photometry and differential viscometry. Peak 1 (MW = 1219697 Da) represents approximately 1% of the total polymers, while peak 2 (MW = 8884 Da) accounts for approximately 99% of polymers. 13C-NMR spectroscopy suggests that glucan sulphate polymer strands may be partially cross-linked. Glucan sulphate (250 mg/kg, i.v.) increased (P less than 0.01) macrophage vascular clearance of 131I-reticuloendothelial emulsion by 42% (P less than 0.01) and in vitro bone marrow proliferation by 46% (P less than 0.05). Glucan sulphate (250 mg/kg, i.v.) increased (P less than 0.05) median survival time of C57Bl/6J mice with syngeneic melanoma B16 or sarcoma M5076. In addition, glucan sulphate immunoprophylaxis increased resistance of mice to challenge with Escherichia coli, Candida albicans or Mouse Hepatitis Virus strain A-59. It was concluded that glucan sulphate activates macrophages, stimulates bone marrow, exerts anti-tumor therapeutic activity, and modifies the course of experimental infectious disease.

7.4. Tumor regression after treatment with aminated beta 1-3D polyglucose is initiated by circulatory failure (Seljelid 1989).

Meth A sarcoma grew progressively when inoculated intradermally in CB6 mice. When the mice were treated on day 7 after inoculation with 10 mg aminated polyglucose (AG) [Bogwald, J., Hoffman, J. & Seljelid, R. Carbohydr Res. 148, 101, 1986], the tumors regressed completely in over 90% of the cases. During the first hours after AG treatment, tumor thymidine incorporation decreased, adenosine triphosphate (ATP) content decreased, and there were indications of circulatory disturbance as shown by decreased deposition of dye (trypan blue) in the tumor tissue after intravenous injection. Histological examination demonstrated a conspicuous thickening of the walls of small tumor vessels, statis of red blood cells, and perivascular collections of mononuclear cells only hours after AG treatment. In thymectomized animals, where regression does not occur after AG treatment [Seljelid, R. Bioscience Reports 6, 845, 1986], there was no evidence of circulatory failure, no tumor diameter reduction, and no decrease in colouring following intravenous injection of trypan blue. On the basis of these findings, we conclude that the early phase of events after AG treatment leading to tumor regression involves a vascular phenomenon that causes circulatory disturbance and necrosis.
The data also indicate that this initial circulatory failure requires the involvement of functional T cells.

8. Description of selected clinical trials

8.1. A "proof-of-concept", double blind, parallel group study comparing the immune modulating and lipid lowering effects of beta-1,3/1,6-glucan (Immutol) with that of placebo (cellulose) in subjects with mild to moderate hypercholesterolemia (Immunocorp AS/Biotec Pharmacon ASA, N-9008 Tromsø, Norway, 2003).

This study was carried out with 140 healthy individuals who according to standards in Norway could be defined as having mild to moderate hypercholesterolemia, but in some other countries were regarded as healthy and not subject to any cholesterol lowering treatment. The primary objective was to record eventual effects on blood CRP levels, since there is a likely link between elevated blood cholesterol and inflammations that may result in increased CRP. In accordance with this hypothesis, the average CRP-level in the study group went down compared to placebo after intake of 0.7 gram micro-particulate beta-1,3/1,6-glucan per day for 8 weeks. But since the majority in the study groups were healthy, most of the analytical figures for CRP were within the healthy range, whereas high values of CRP dropped off in the study group compared to placebo. Studies with individuals having inflammations and hence elevated CRP-levels are therefore logical follow-up of this combined safety/"proof-of-concept" study.

8.2. Randomized phase I/II trial of a macrophage-specific immunomodulator (PGG-Glucan) in high-risk surgical patients (Babineau et al. 1994b)

A double-blind, placebo-controlled, randomised, single centre phase I/II trial of PGG-glucan was conducted in high-risk surgical patients. 34 patients at high risk of postoperative infection undergoing major abdominal or thoracic surgery were randomised in a 2:1 ratio to receive PGG-glucan or placebo. 0.5 mg/kg of PGG-glucan and saline placebo were administered in multiple, sequential doses by intravenous infusion (continuous infusion for 1 hour) 12-24 hours before surgery, 1 to 4 hours before surgery, 48 hours after surgery, and 96 hours after surgery. The patients were evaluated before surgery and until their discharge from the hospital. In addition, long-term follow-up was performed 4 and 8 weeks postoperatively. Statistical analyses were done using the chi square test and analysis of variance.

No adverse drug experiences associated with PGG-glucan infusion were observed.

Patients who received PGG-glucan had significantly fewer infectious complications (3.4 infections per infected patient vs. 1.4 infections per infected patient, p=0.05), decreased intravenous antibiotic requirement (10.3 days vs. 0.4 days, p=0.04), and shorter intensive care unit length of stay (3.3 days vs. 0.1 days, p=0.03). The hospital length of stay was shorter for patients in the active group, but this did not reach statistical significance. Even if the number of infections was reduced in the PGG-glucan group, the number of patients infected did not differ between the two groups. None of the in vitro tests of microbicidal activity of neutrophils and macrophages against S. aureus, E. Coli and C. albicans were statistically significant, but the monocytes from patients treated with PGG-glucan showed a trend toward increased microbicidal killing activity against S. aureus and C. albicans.

8.3. A phase II multicenter, double-blind, randomized, placebo-controlled study of three dosages of an immunomodulator (PGG-Glucan) in high-risk surgical patients (Babineau et al. 1994a)

A double-blind, placebo-controlled, randomised, multicentre phase II trial of three dosages of PGG-glucan was conducted in high-risk surgical patients. 67 patients scheduled for major noncardiac thoracic or abdominal surgery were randomised in a 1:1:1 ratio to receive saline placebo or PGG-glucan at a dose of 0.1 mg/kg, 0.5 mg/kg, 2.0 mg/kg. After six weeks' administration of a dose of 2.0 mg/kg, this dose was reduced to 1.0 mg/kg based on minor adverse experiences in healthy volunteers who received a dose of 2.25 mg/kg. The patients received multiple, sequential doses by intravenous infusion (continuous infusion for 1 hour), one dose was administered 1 to 6 hours before surgery and three doses were administered after surgery (within 4 hours, at 48 and 96 hours after surgery). The patients were evaluated before surgery and until their discharge from the hospital. In addition, long-term follow-up was performed 4 and 8 weeks postoperatively. Statistical analyses were performed using the chi square test with Yates' correction.

Of the 67 patients enrolled, 64 were evaluable. Two patient withdrew before study completion owing to adverse experiences: An episode of hypertension, diaphoresis and nausea following the third dose of 0.1 mg/kg and an episode of maculopapular rash on the abdomen and trunk following the second dose of 2.0 mg/kg. The incidence and severity of adverse events was comparable in all groups. No serious adverse events were considered to be related to PGG-glucan administration. A dose-response trend with regard to infection incidence among patients who receive PGG-glucan was observed, although this did not reach statistical significance. Serious infections occurred in four patients who received placebo and in three patients who received PGG-glucan at a dose of 0.1 mg/kg. However, only one patient who received PGG-glucan at a high dose had a serious infection.

8.4. Effect of PGG-glucan on the rate of serious postoperative infection or death observed after high-risk gastrointestinal operations (Phase III study) (Dellinger et al. 1999)

A double-blind, placebo-controlled, randomised, multicentre trial with PGG-glucan after high risk gastrointestinal operations was conducted at 39 medical centres in the USA. 1249 patients scheduled for a gastrointestinal procedure lasting 2-8 hours were randomised to receive saline placebo or PGG-glucan 0.5 mg/kg or 1.0 mg/kg, stratified into colorectal (planned procedure involving incision in the colon or rectum) and non-colorectal (no incision planned) strata. The patients received 4 doses of study drug by intravenous infusion, one dose was administered within 12 hours prior to surgery, and three doses were administered after surgery (within 4 hours after the first dose, at 48 and 96 hours after start of surgery). All patients received antibiotic prophylaxis according to protocol.

1177 patients constituted the intent-to-treat population. Treatment effects were tested using a Cochran-Mantel-Haenszel c2 test for differences in mean scores adjusting for investigator and stratum. There was no significant difference in the primary end point events, serious infection and/or death, between any of the groups. When analysing the treatment effects separately for the
collected and non-colon strata, there was a reduction in the incidence of infection and death in the PGG-glucan groups (36% incidence) compared to the placebo group (22% incidence) in the non-colon stratum, although this was not statistically significant. Examination of the colon stratum failed to demonstrate any treatment effect.

44 patients died within 30 days, with no significant differences among the treatment groups for patients who died with or without infection. PGG-glucan was well tolerated. There was a slight increase in fever, hypertension, nausea, and vomiting seen primarily in the 1.0 mg/kg dose group. Serious adverse events were common in all groups, reflecting the serious operations and high-risk nature of the patients, and were not different between groups.

8.5. Infection prevention in patients with severe multiple trauma with the immunomodulator beta-1,3 polyglucose (glucan) (Felippe et al. 1993).

To study the possible prevention of pneumonia and sepsis in patients with multiple trauma with head injury, beta 1,3-glucan was used prophylactically in patients who were not infected within 48 hrs of admission to the hospital. Forty-one patients were stratified using the Trauma Score, included in the double-blind controlled trial and randomised into a control group (n=20) or a glucan group (n=21). The first nine patients received 30 mg glucan i.v. every 24 hrs, with a mean of 312±250 mg per patient during 8.8±5.0 days. The next 12 patients received 30 mg glucan i.v. every 12 hrs, with a mean of 685±376 mg per patient during 11.6±6 days. Significant decreases (55.0 to 9.5%) in the pneumonia incidence, the sepsis incidence (35.0 to 9.5%), and the mortality rate to infection (30.0 to 4.8%) were observed in patients treated with glucan. The overall mortality rate, cerebral deaths excluded, was 42.1% in the control group and 23.5% in the glucan group.

Weakness and tiredness was observed in 17 of the patients, and headache, body pain, stomatitis and pharyngitis in one of the patients each. The side effects were rare, light and did not require interruption of treatment, but a slight reduction of doses.


A prospective, randomised, double-blind study was conducted to evaluate the efficacy of soluble glucan in 38 trauma patients undergoing surgery (exploratory laparotomy or thoracotomy for trauma). Glucan 50 mg/m2 (n=21) or saline placebo (n=17) was given iv. daily for 7 days after operation. All patients received prophylactic antibiotics. The total morbidity rate, but not the mortality rate from sepsis, was statistically significant reduced in the glucan group (0% vs 29%, p<0.05). The glucan patients had a transient significant increase in IL-1 (143.4%±19.3% vs 78.6%±11.7%, p<0.05), but there was no difference in the serum TNF levels of placebo or glucan treated patients.


The efficacy of a beta-glucan/collagen matrix (BGC), which combines the carbohydrate beta-glucan with collagen in a meshed reinforced wound dressing, has been evaluated in a retrospective chart review of 225 consecutive pediatric burn patients. Of the 225 charts reviewed, 43 patients (19%) were treated with BGC as a primary wound dressing, and 130 patients (58%) received standard treatment (daily dressing changes with silver sulfadiazene or bacitracin ointment) without the use of BGC or split-thickness skin grafting. Statistical comparisons of the treatment groups did not reveal any difference in terms of days to healing. Thirty-four patients (79%) had the BGC remain intact while the wound healed underneath, with good cosmetic results, minimal analgesic requirements, and no need for repetitive dressing changes, while nine of the patients (21%) had the BGC removed before wound healing due to progression of the burn or nonadherence. BGC is maintained to markedly simplify wound care and to decrease post-injury pain. BGC is also believed to act as an effective barrier against bacterial contamination.

9. Skepticism to beta-1,3/1,6-glucan

The possibility of using beta-1,3/1,6-glucan in human medicine has been met with skepticism, possibly because some results of clinical investigations have not been reproducible. This is, however, most probably due to discrepancies in dosage and application methods and also because of poorly characterized and defined chemistry of the glucan preparations used. It is not enough to refer to a substance as beta-1,3-glucan. One must understand the molecular structure to be able predict any effect. For example, it is a presupposition for macrophage activating ability and biological effect of a beta-1,3/1,6-glucan that the molecule has branches in 6 positions on the beta-1,3-glucan chain. In addition, the effect of the beta-1,3/1,6-glucan varies with the frequency and length of these side branches (see 4.1).

Another cause for skepticism can be that scientists/researchers, being used to the ideas from specific immunology, simply have not believed in immunotherapy with substances that are targeted on innate immune mechanisms. A change has occurred in recent years, however, as this "Ancient system gets new respect" (Gura 2001).

10. Conclusion

Important discussions are underway on the fight against serious infections and strategies for developing new anti-infection processes. In the present summary monograph we have pointed to the experience and future potentials in adopting the strategy to stimulate the body's own innate defense system in a way which parallels early steps in the natural course of an infection. This strategy has been very successful in health management of farm animals, using beta-1,3/1,6-glucan preparations from yeast as prophylactic agent in feeds. Use of beta-1,3/1,6-glucan preparations as a dietary supplement to enhance immunity in humans is a practice that can be justified not only from the overwhelmingly positive results with animals, but also from human studies and a scientific foundation that show that beta-1,3/1,6-glucans:

- Have molecular structures that are alien to the animal kingdom and therefore are trustworthy "alarm signals" for mobilizing the immune system to counter infections;
- Activate phagocytic cells by a mechanism that has been described in great detail at a molecular and cellular level;
- Cause enhanced protection against virus, bacteria, fungi and parasites by mobilizing innate immunity when administered onto mucous surfaces or as a dietary supplement;
- Are non-toxic and do not induce antibody production against itself;
- Enhance specific antibody production against vaccine antigens and do not induce tolerance to vaccine antigens;
- Stimulate wound healing and repair of damaged cells;
- Counteract inflammations induced by infections.

In addition, beta-1,3/1,6-glucans may be the supplement of choice for modern people living in urban societies to compensate for eventual insufficient or inadequate exposure to microbial challenges, and thereby possibly counteract immune related disorders that are more and more common in these societies.
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