The world of β-glucans – a review of biological roles, applications and potential areas of research

Thesis for the requirement of Master of Science – Medical Biology

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The effort herein is dedicated to my darling brother Wajid, the sweetest little brother in the whole wide world, a true charm ☺

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ABSTRACT

Among many known and tested immunomodulators, polysaccharides isolated from various natural sources occupy a prominent position. An important group of these polysaccharides is represented by the homopolymers of β-glucose, called β-glucans. Their very low-to-negligible toxicity and immunomodulating effects show the promise it has in the therapy of a variety of infectious and cancer illnesses. Nowadays, the popularity of Glucans as food additive and a disease-modifying agent is increasing. Here, a review of the various biological roles, applications and potential areas of β-glucan research is presented. Also, a short introduction to current work by the Glucan group in Tromsø is provided. The goal has remained to understand and aptly present the diverse roles of β-glucans and pin-pointing the prospective areas of research, both on the receptor / biochemical level, and in clinical research. It seems that with the tide of modern medical research, gradually, β-glucans will take the position they deserve in diagnostic and preventive medicine.

INTRODUCTION

INNATE IMMUNITY:

Immunity, why and how: With Immunity's definition as body's resistance to an agent (a microbe, a cancerous cell, etc.) that may cause a derangement in body's homeostasis, many questions arise in one's mind. What gives the immune system the capability and mechanistic adaptability to drive a network of specialized cells and organs toward defense against infectious agents and cancerous cells? And how its malfunction could unleash a torrent of diseases, from allergy to arthritis to cancer to AIDS?

One of immune system's remarkable characteristics is distinguishing between "self" and "non-self". The Journal of Clinical Investigation defines [1] "self" as, "the antigens expressed on the surface of normal human cells, which are ineffective at triggering immune responses against themselves." In this context, the "non-self" could be defined as mutated self-antigens (as in cancer), antigens expressed on the surface of infectious agents (bacteria, virus, fungi, parasites, etc.), and chemicals deemed hazardous by the immune system. Indeed, immune system is remarkable in the specificity it exercises in recognizing many millions of distinctive non-self molecules.

The two major divisions of human immune system, the innate and the adaptive, provide a comprehensive coverage of microorganisms and cancer cells. The "innate" immune system is our first line of defense, and the "adaptive" system affords protection against re-exposure to the same pathogen. While both systems have cellular and humoral components, the innate immune system also possesses anatomical barriers to infection described later. And they complement each other too: the innate being the capturer and processor of foreign antigens, and the adaptive taking heed from the former, spearheading the development of antibodies against an antigen the body has already encountered.

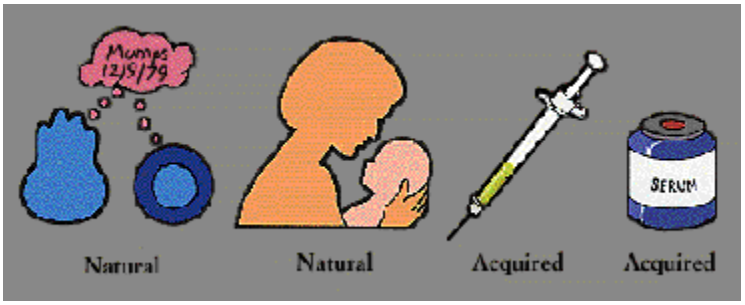

Fig. 1: The types of immunity and the methods to boost them [*1]

Litt om "Innate" immunity:

The innate immunity has the job of immediate recognition of antigenic molecules that are prevalent and conserved throughout a particular invading species or compound. Such molecules are uniform structures essentially embedded in the invader's architecture, unaltered by mutations or selection, and can come from any foreign invader such as viruses, bacteria, fungi, parasites, pollens, foods, drugs, etc. The antigens could be proteins or oligosaccharides, and act as markers on microbial surface to help identify the cell as self or non-self, identify the type of cell and stimulate immune cell responses (T cells) and the production of antibodies.

Male et al [*2] divide innate immune system in three components: the anatomical barriers, the humoral component, and the cellular component.

The anatomical barriers are the first line of defense and provide mechanical hindrance to invading hazardous agents. They include the skin and internal epithelial layers, the peristaltic intestinal movements and the oscillation of bronchopulmonary cilia. Associated with these protective surfaces are chemical and biological agents. The *Chemical factors* in the anatomical barriers include fatty acids (in sweat), lysozyme and phospholipase (tears), saliva and nasal secretions, low pH gastric secretions, Defensins (lung & gastrointestinal tract), and surfactants in the lung acting as opsonins (promoting phagocytosis). The *Biological factors* are the normal flora of the skin and the gastrointestinal tract (GIT), which secrete toxic substances to prevent the colonization by pathogenic bacteria or by competing against them for nutrients.

The Humoral barriers to infection come into play when anatomical barriers are breached. The humoral response includes 'secretory' factors found in serum or at the site of infection and include: complement (proteins) system, the activation of which can lead to raised vascular permeability, recruitment of phagocytic cells, and lysis and opsonization of bacteria; coagulation system, the contribution of which lies in its ability to increase vascular permeability, to act as chemotactic agents for phagocytic cells, and having a direct antimicrobial action in some of its products (e.g., β-lysin, a platelet protein); lactoferrin and transferring, which devoid bacteria of an essential nutrient, iron; interferons, which limit viral replication; lysozyme, which breaks down bacterial cell wall; and interleukins, especially IL-1, which induces fever and promotes opsonization by causing a rise in Acute Phase Protein levels.

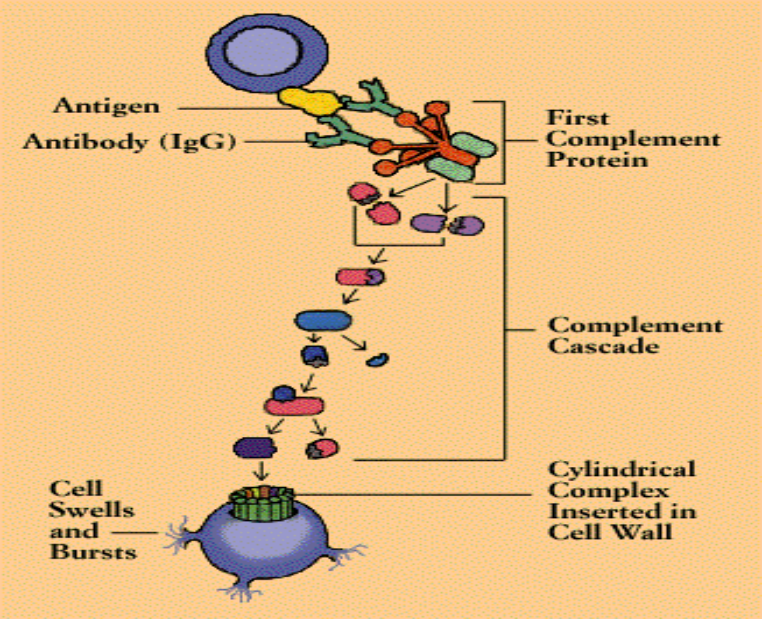

Fig 2: Complement proteins in action → swell & burst [2]

The Cellular barrier to infection are posed by White Blood Cells (WBCs), the major component of immune system cells. Among the most prominent ones are: neutrophils, with a characteristic CD66 marker on their surface, which cause phagocytosis and intracellular killing but may cause collateral tissue damage ('pus' formation); macrophages, with CD14 as their surface marker, which cause intra and extra-cellular killing of infected or altered-self target cells plus act as antigen-presenting cells; Natural Killer (NK) and Lymphokine Activated Killer (LAK) cells, which non-specifically kill virus-infected or tumor cells; eosinophils, which cause allergy responses and kill certain parasites. The recruitment of eosinophils and macrophages to the site of infection is the main line of defense in the innate immune system.

Also, the cellular barrier includes non-specific killer cells such as NK, LAK and K cells. *(NK)* cells are a special sub-type of cytotoxic lymphocytes identified by presence of CD56 and CD16 and lack of CD3 cell surface markers. They kill virus-infected and malignant target cells (Fig 6), and are more potent when stimulated with IL-2 and IFN-γ, which transforms them to lymphokine-activated killer (LAK) cells. NK and LAK attach killer activating ligand (KAL) present on the diseased cell's surface to mark it as diseased and 'destined to be killed'. On the other hand, if an inhibitory ligand (MHC-class I molecule, a sign of normal cells) is present on the cell's surface, it binds the Killer Inhibitory Receptor (KIR) on NK and LAK cells and spares itself from destruction. *Killer (K) cells* mediate antibody-dependent cellular cytotoxicity (ADCC) in which an antibody acts as a link to

bring the K-cell and the target cell together via an Fc-receptor on K cell surface. Killer cells with Fc receptors are NK, LAK, and macrophages (Fc receptor for IgG) and eosinophils (for IgE antibodies).

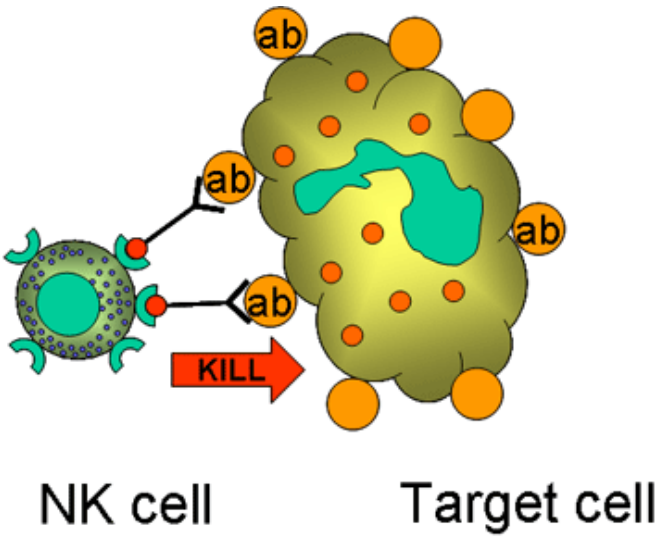

Fig. 6: Killing of opsonised target by by NK cell [*2]

Phagocytosis and intracellular killing:

The concept of 'phagocytosis' is that a neutrophil or a macrophage engulfs a foreign organism, trying to 'even' its harmful effect. One can imagine neutrophil being a motile, multi-lobed cell containing granules, which upon fusion with the engulfed substance, pours cationic proteins, proteolytic enzymes, cathepsin G, lysozyme and characteristically, myeloperoxidase, lactoferrin, and B12-binding protein into the engulfed organism. Macrophages (monocytes in circulation), however, are tissue-resident cells with kidney-shaped nucleus and no granules, but lysozymal activity. Action of either of these cells culminates in bacterial cell wall degeneration and cell bust.

Phagocytosis of foreign organisms begins when an "exogenous" inflammatory mediator signal immune cells about microbial presence in the surrounding. Such "exogenous" mediators are called endotoxin or lipopolysaccharide (LPS), and are present on microbial surface. They are sensed by toll-like receptors (TLRs) on human immune cell surface, from where a cascade of intracellular and extracellular events begins that causes the mounting of an immune response (inflammation).

The immune response progresses when phagocytes send SOS signals at the site of infection or inflammation for enhanced expression of cell adhesion molecules (ICAM-1 & selectins) on vascular endothelium. This will cause diapedesis of circulating phagocytes toward the site of action (Fig 4). These SOS signals play a role also in chemotaxis and activation of phagocytes.

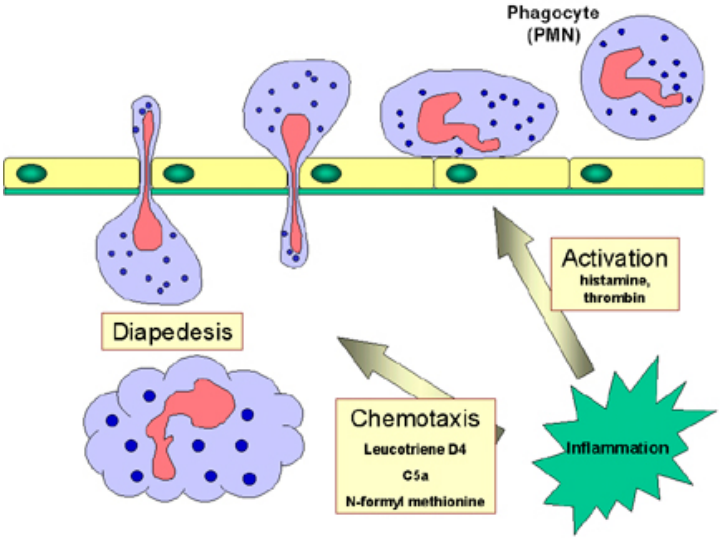

Fig. 4: Chemotactic response to inflammatory stimulus [*2]

Major phagocytes include macrophages and neutrophils, which have slightly different mechanism of phagocytic action. Macrophages are "scavengers", which rid the body of worn-out cells and other debris. By "presenting" an antigen to T cells having first digested and processed it, macrophages play a crucial role in initiating the immune response. They are also secretory in nature, churning out a range of chemicals including enzymes, complement proteins and regulatory factors such as interleukin-1, which have a role in development of immune response. Neutrophil, another critical phagocyte, is a granulocyte as well. It contains granules filled with potent chemicals meant to destroy microorganisms and play a key role in acute inflammatory reactions.

Antigen-presentation and phagocytosis is characteristic also of other cell types such as B cells and dendritic cells (irregularly shaped WBCs found in the spleen and other lymphoid organs). Dendritic cells typically have long threadlike tentacles that entangle lymphocytes and antigens and then process and present the antigens on its surface. B-cells are lymphocytes involved in memory and antibody production. Many other cell types can also be recruited to present antigens to lymphocytes upon proper stimulation.

A variety of receptors (Fig. 5 and Fig. 9) on phagocytic cell surface interact with infectious or cancerous cells. Among the significant ones are Fc receptors (binding Fc-region of the antibody), complement receptors (for C3b component), scavenger receptors (for polyanions on bacterial surfaces) and Toll-like receptors (the so-called Pattern Recognition Receptors, PRRs, which recognize pathogen associated molecular patterns, PAMPs). The receptor interaction causes activation of phagocytes (respiratory burst) which leads to phagocytosis and the release of inflammatory cytokines (ILs and TNF).

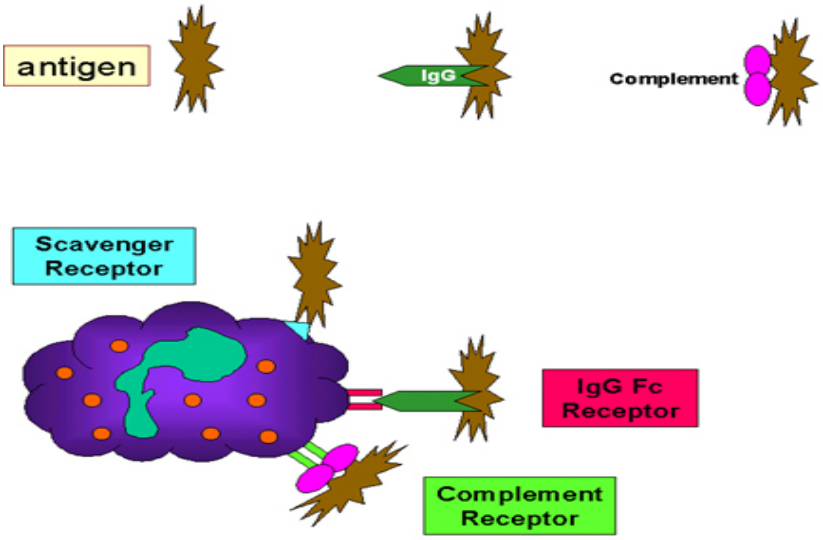

Fig. 5: Adherence of bacteria via different receptors [*2]

Respiratory burst is an important component of phagocytosis. It occurs with an increase in glucose and oxygen consumption that causes “oxygen-dependent” intracellular killing of microorganisms or the potential cancerous cell. This is separate from the lethal effect of granules or lysosomes, which is "oxygen-independent".

Oxygen-dependent killing is interesting in the aspect that it could be two-tier: myeloperoxidase-independent, via toxic oxygen elements such as superoxide anion (O^{2-}), H_2O_2, singlet oxygen and hydroxyl radical (OH•), or (myeloperoxidase-dependent), via Hypochlorite which is generated with the release of myeloperoxidase into the phagolysosome. The phagocytes themselves are protected from these toxic intermediates via dismutation ($O^{2-} \rightarrow H_2O_2$) and catalation ($H_2O_2 \rightarrow H_2O$). *Oxygen-independent* intracellular killing mechanism uses cathepsin, lysozyme, lactoferrin, and hydrolytic enzymes. Although less efficient, this mechanism is especially useful in patients with genetic defects in the oxygen-dependent killing pathways.

β-GLUCANS:

Origin and Structure:

β-glucans are glucose polymers, recognized as the effective ingredients in fungal and certain bacterial cell walls. About half the mass of the fungal cell wall consists of β-glucans. Natural products containing fungal β-glucans have been consumed for probably thousands of years, especially in China and Japan for their role in improving general health. In recent years, β-glucans have been noted as potent stimulators of mammalian immune system, and now are used clinically in China and Japan.

Among the products with B-glucans, Zymosan is a very potent immunostimulator that has been widely used in research. It is a mixture of proteins, lipids and polysaccharides isolated from the cell wall of Saccharomyces cerevisiae and was first prepared and investigated in 1941 by Pillemer et al. Carrying on the work, Di Luzio and coworkers in 1970s, pioneered the immunological research on the function of purified β-glucans.

β-glucans are present in abundance in cereals such as barley and oats [2-5] and their purification methods are rather simple. Structurally, they have a linear backbone of D-glucose in β-1,3 linkage with side branches in β-1,6 linkage at various intervals (Fig. 6). The presence of side branches in the intermediate layer of the cell wall imparts shape and rigidity to the cell. The molecular structure also depends also on the source and method of isolation with differences in the distribution and length of side chains. Solubility of β -glucans is associated with the degree of polymerization (DP), being completely insoluble in water when DP > 100.

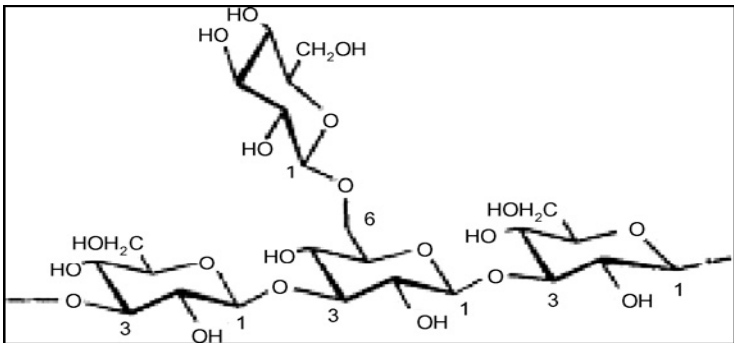

Fig. 6. Structure of (1,3) β -glucans with ramifications β (1,6) [6]

Other forms of β-glucans also exist, with β(1,3) and β(1,4) linkages (Fig. 7).

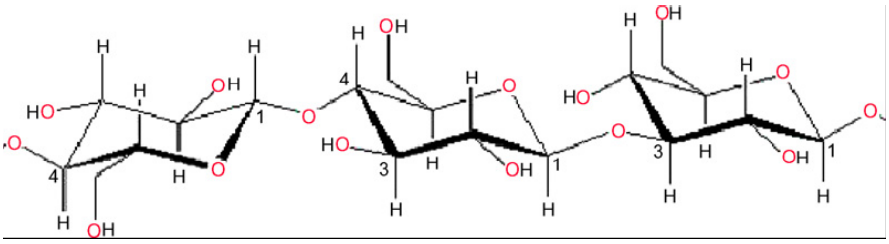

Fig. 7. Structure of (1,3)- β -glucans with ramifications β (1,4) [6]

They can be classified according to solubility properties [5]: alkali-insoluble, acetic acid insoluble (1,3); alkali-soluble (1,3); and highly branched (1,6). Most β -glucans are insoluble however, thereby limiting their application in *in vitro* experimental studies.

Glucans of interest:

Some naturally occurring glucans are of particular clinical interest. The noteworthy 'natural' β-glucans are Lentinans, Schizophyllan, PSK (Krestin). Lentinan is mushroom-extracted and has a triple helix structure with five (1-3)- β-glucose linear residues and two (1-6)- β-glucopyranoside side branches. Schizophyllan, from the mushroom of *Schizophyllum commune*, has β-glucopyranosyl 1-6 linkage every 3rd or 4th-interval between the 1,3 units. It also has a triple-helix structure. PSK (krestin) is composed of 25–38% protein residues and is a 1-4-β-glucan with 1-6-β-glucopyranosidic lateral chains. Obtained from mushroom *Coriolus versicolus*, it has a molecular weight of 94 kDa, the least among the 'natural' glucan types [3-5].

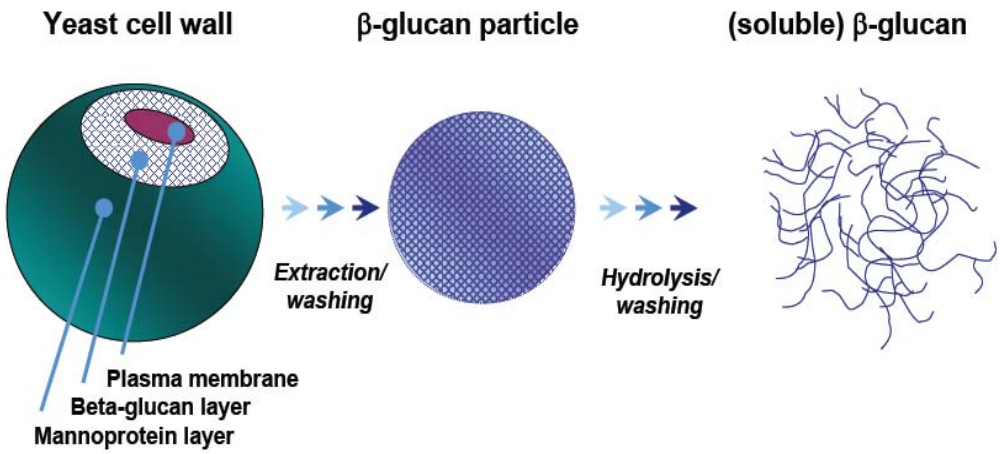

Fig. 8. Extracting a 'soluble' glucan. Figure courtesy Biotec Pharmacon, ASA

The yeast-extracted glucans have been subdivided into soluble (SBG) and non-soluble (NBG) types. The soluble type is administered parentally, while non-soluble type can only be given orally. An insoluble (1,3/1,6) β-glucan derived from baker's yeast differs from its soluble (1,3/1,4) counterparts in molecular structure and shows higher biological activity owing to the "branching" in its structure. Due to this structural difference, soluble and insoluble β-glucans have different application, mode of action, and overall biological activity. With the use of either, it has been demonstrated that β-glucan may play important role in many functions of the body and affects many immunity-related disorders. They increase neutrophil chemotaxis and adhesion, synergize with myeloid growth factors to enhance hematopoiesis and mobilize peripheral blood progenitor cells *in vivo*. Also, the glucans have been shown to directly stimulate committed myeloid progenitor cells and improve survival and hematopoietic regeneration in irradiated mice. Alongside, they amplify phagocytic killing of opsonized tumor cells and combine with monoclonal antibodies to increase their tumoricidal activity.

Role of Glucans in health promotion and therapeutics:

The *General effects on health* are a special function of immunomodulatory properties of β-glucans. *In vitro* and *in vivo* studies show that Glucans have a stimulatory effect on innate immune system against bacterial, viral, fungal and parasitic infections [7-10]. Activation of macrophages, T-helper and natural killer (NK) cells, and the promotion of differentiation and activation of T lymphocytes for the alternative complement pathway [9] have been proposed behind this. β-glucans are also called the modulators of humoral and cellular immunity [10-12] and a modulator of the anti-inflammatory response with similar outcome as interleukin mediators[13]. Candida albicans (yeast) β-glucan has been shown to activate macrophages and induce production of interleukin-6 (IL-6) and tumor necrosis factor (TNF) *in vitro*, promoting vascular permeability and stimulating the classic complement pathway [12]. β-glucans of higher molecular weight, like the one obtained from *mycelium*, have also shown similar effects [14].

Since β-glucans tend not to be degraded by human enzymes, they have a natural nutritional fiber property. A high-fiber diet has demonstrated protective hypocholesterolemic effect [4] reducing the risk of chronic diseases such as arthritis and heart disease. β-glucans do this by increasing intestinal viscosity and reducing cholesterol absorption, thereby promoting cholesterol excretion and imparting a hypocholesterolemic effect

[15]. This has prompted the U.S. Food and Drug Administration (FDA) to approve some patents of β-glucans to be sold over-the-counter, for treatment of hypercholesterolemia.

Therapeutic effects of β-glucans are multifarious, ranging from general health benefits to specific therapeutic benefits. Upon reductive amination, the glucans extracted from *Poria cocos* and *Pleurotus tuber-regiu* show antimicrobial and especially anti-viral effect, as one study suggested [16]. Upon sulfation, the glucans act as anticoagulant, especially the ones extracted from bacterium *Alicaligenes faecalis*, and a mushroom, *Parmotrema mantiqueirense* [17, 18]. Comparing the cationic and native (untreated) β-glucans, the latter was shown to inhibit bacterial growth by ~35%, while the cationic one showed 80% inhibition. Such properties can be attributed to increased solubility owing to increased ion density. This implies that after undergoing commercial treatment (e.g, amination) antimicrobial effects of β-glucan can be promoted.

Compound of potential genetic mutagenic nature could be antagonized by usage of β-glucans, preventing the hazards of developing many illnesses such as cancer. The barley β-glucan shows protective effect against methyl methanesulfonate (MMS)-induced damage in the CHO-K1 (hamster ovary) cell line, which prevents abnormalities in drug metabolism [19]. It also has a protective effect against genotoxicity and cytotoxicity from anti-cancer drugs such as cyclophosphamide, adriamycin and cisplatin. β-glucan, when administered prior to Methotrexate (a cytotoxic anti-rheumatic and anti-cancer agent) prevents organ damage resulting from Methotrexate-mediated depletion of GSH enzyme in ileum, liver and kidney [20]. β-glucans of different origin are found to be potent anti-oxidants as well, preventing damage by H_2O_2 and other reactive oxygen species [21, 22].

Among the latest studies on the diagnostic and therapeutic potential of β-glucans, a few are as follows.

In 2004, a group of researchers in Japan published a paper [23] on the potential use of β-(1,3) glucan in the diagnosis and treatment of keratomycosis, a fungal infectious disease of the cornea of the eye. Using animal model of keratomycosis, they proposed a new method of detecting β-(1,3) glucan in the tears of the experimental animals. This species-unique β-glucan would be the component of fungal cell wall, and therefore this technique hints at the causative infectious agent. The study also showed good efficacy of topical application of Micafungin, an antifungal agent that inhibits the activity of β-glucan synthase. A couple of years later, Pazos et al. [24] published a clinically-relevant study on the diagnostic potential of detection of β-(1,3) glucan and antibodies to Candida albicans germ in invasive candidiasis in neutropenic adult patients.

With regards to therapeutics, within the last couple of years two studies [25, 26] are especially of note. One of them on the potential antioxidant activity of β-(1,3) glucan and protein fractions from Saccharomyces cerevisiae cell walls, and the other on therapeutic potential of various β-glucan sources in conjunction with antitumor monoclonal antibodies in cancer therapy. Both studies highlight the role β-glucan can play, not just in preventive medicine, but also in diagnostics and therapeutics of major illnesses such as infectious diseases and cancer.

Table 1
Structure, origin and biological activities of β-glucans

Structure	Source	Effects
β (1 → 3) (1 → 6)	*Saccharomyces cerevisae*	Antiparasitic
		Antibacterial
		Antiviral
		Antifungal
		Antimutagenic/antigenotoxic
		Antitumoral
		Hematopoietic stimulator
		Mitogenic
	Candida albicans	Imunostimulating activity
	Poria cocus	Antitumoral
	Agaricus blazei	Cytokine induction
		Antimutagenic/antigenotoxic
		Inhibition of CYP450 isoenzymes
		Antitumor
	Lentinus edodes	Inhibition of CYP450 isoenzymes
		Antitumor
	Schizophyllum commune	Antitumor
	Coriolus versicolor	Antitumor
β (1 → 3) (1 → 4)	Oat	Antimicrobial
		Antiparasitic
		Hypocholesterolemic
		Anti-thrombotic
	Barley	Antimutagenic

Table 1. Summary of various functions of β-glucans, 1-3, and 1-4 and 1-6 [6]

Current interest in Glucans:

Basic research on the mode of action of the different types of β (1,3-1,6)-glucans at cellular level has been the foundation for scientific experiments with whole animals and clinical trials. Many studies have confirmed that β-glucan enhances overall disease resistance and improves health and performance, with per oral administration either as feed formulations or as stand-alone products onto mucous surfaces. These studies have shown that pure β-glucan is a non-toxic modulator of various immune processes.

Insoluble and soluble β-glucan and some clinical trials: Currently, there has been interest shown in the role of β-glucan in tumor regression and protection from various infections and to attenuation of ischemia reperfusion injury [27, 28]. Also, the effectiveness of glucans obtained from different sources has been under the spotlight. In a study on the efficacy of various β-glucans, the bioavailability of three different soluble ones was found to be as low as 0,5-4,9% in circulation, whereas a water-insoluble microparticulate β-glucan was not detected in the systemic circulation after oral ingestion [29]. On the other hand, some have reported immunomodulating effects of soluble[30-35] as well as microparticulate-insoluble β-glucan [36, 37] following oral administration. In more experimental studies, oral β-glucan has also been shown to protect against lipopolysaccharide-induced shock (from infectious agents) in rodents [30-35] , and lower release of myocardial enzymes in the postoperative period following coronary artery bypass grafting, indicating a possible cardio-protective effect [31]. Another study highlighted the role of microparticulate β-glucan in protecting against renal ischemia reperfusion injury [32].

In our experiments at UiT, we use the NBG and SBG samples provided by Biotec Pharmacon, a company of bio-products based in Norway. Their products have shown beneficial applications in general infection prophylaxis especially HIV-AIDS and tuberculosis, immune therapy of cancer and pre-op and post-op repair of damaged heart tissue in cases of myocardial infarction. In collaboration with Biotec Pharmacon, the β-glucan group at UiT has some clinical development and R&D programmes with cooperating academic and scientific institutions in Norway and abroad, particularly in South Africa and USA. Among other focuses, we want to investigate the receptors involved in uptake of β-glucan in the gut. Dectin-1 has previously been described in leukocytes and dendritic cells [38], but has not been extensively studied in epithelial cells. So for a while, one subgroup of the UiT Glucan group has been working on epithelial cell lines to investigate potential receptors for β-glucan.

RECEPTORS IN INNATE IMMUNITY:

Introduction: Characteristic microbial products are recognized by scavenger cells of innate immunity. This recognition is via receptors and leads to intracellular signaling mechanisms to give an efficient cellular response. Such responses range from phagocytosis and related intracellular processes essential for handling ingested microbes to release of a broad range of mediators. The mediators are involved in the efferent arm of the innate immune response and include cytokines, chemokines, antimicrobial peptides, lysozyme, BPI, lactoferrin, proteases, lipases, glycosidases, superoxides, nitric oxide, and many others. But first, we will elaborate a bit on the basis of receptor-ligand interaction in innate immunity.

In order to recognize and respond to the antigens that are their specific targets, both B cells and T cells carry special receptors on their surface. The *B cell receptor* is a homologue of the antibody secreted by that particular B cell itself. When a B cell encounters a matching antigen, this antibody-like receptor allows the B cell to interact with it. The *T cell receptor* is more complex. It is made of a pair of chemically linked chains with variable and constant regions and requires signaling and anchoring cell surface molecules (CD3) in order to work. It cannot recognize antigen in its natural state. The antigen must first be broken down, and the fragments bound to an Major Histocompatibility Complex (MHC) molecule, by an antigen-presenting cell. Helper T cells (CD4 cells) look for antigen bound to a class II MHC molecule on Antigen Presenting Cells (APCs) like macrophages and B cells. The cytotoxic T cells (CD8), however, respond to antigen bound to MHC class I molecules which can be found on almost all body cells. This is how a T-cell receptor molecule forms a three-way complex with its specific foreign antigen and an MHC protein.

The major antigen receptor, called "α/β" for its two chains, is found on most CD4 and CD8 cells. Another type is "γ/δ" found on a distinct subset of T-cells, but with a yet-undiscovered function. Both receptor types work in conjunction with CD3, a signal transduction module made up of various chains.

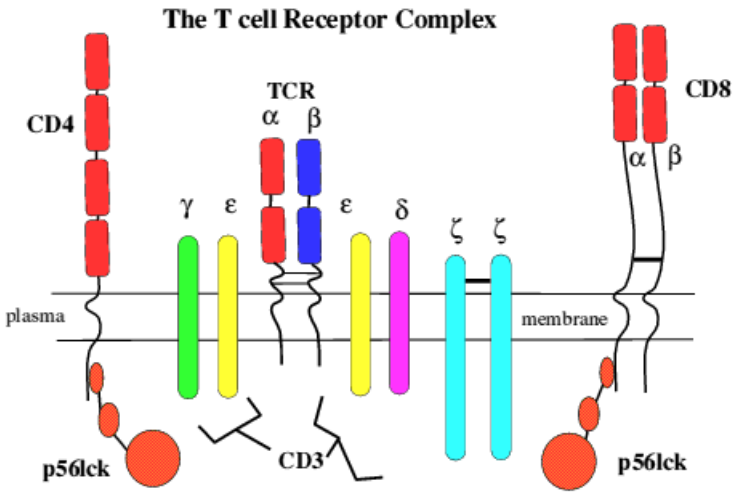

Fig. 9: The T-Cell Receptor (TCR) complex [*1]

The peptide antigen binds to T-cell receptor (TCR) in association with the MHC gene products CD3, and this leads to a series of intracellular biochemical events culminating in the transcription of new genes and cellular activation. The biochemical events start with the activation of one or more tyrosine kinases that phosphorylate first the CD3 chains themselves, and subsequently other substrates. After tyrosine kinase activation and TCR engagement, activation of serine/threonine kinases takes place which causes activation of the GTP-binding protein p21ras and activation of transcription factors for receptors and growth factors such as interleukin-2 (IL2). The CD4 and CD8 co-receptors bind a tyrosine kinase (p56lck) via their intracytoplasmic tail which plays a critical role in T cell signaling.

The immediate recognition of a foreign substance by innate immune cells is dependent on scavenger receptors. The revolution in innate immunity research in the decade led to the discovery of the mammalian Toll-like receptors and new scavenger receptors such as LOX-1 and Dectin-1 and to the rapidly increasing insight in their functions. These receptors are unique in terms of the existence of germline-encoded molecules called pattern recognition receptors (PRRs). The PRRs recognize specific pathogen-associated molecular patterns (PAMPs) commonly present in microbes but not in host. Upon detection of PAMPs, some PRRs trigger an inflammatory response leading to efficient destruction of the invading pathogens. They can be membrane bound such as the type A, B and C scavenger receptors, integrins, Toll like receptors and C type lectin receptors, or they can be intracellular, the prime example of which are the newly discovered nucleotidebinding oligomerization domain (NOD) proteins. Unlike the receptors of adaptive immune system,

the PRRs do not undergo somatic mutations, so can be conserved throughout a species population. The major scavenger receptors can be classified as the table below.

Receptor	Microbial ligand
Toll like receptors 1-9	Endotoxin, lipoteichic acid, peptidoglycan, dsRNA, ssRNA, flagellin, CpG DNA and many others
CD14	Endotoxin, peptidoglycan
CD36 + TLR2	Di-acyl lipopeptide and lipoteichoic acid
Scavenger receptors	Endotoxin, lipoteichic acid, bacterial wall
MARCO	Bacteria
Dectin-1	Beta-glucan
Mannose receptor	Mannan
DC-SIGN	ManLAM
FMLP receptor	N-formylated bacterial peptides

Table 2: Selected receptors of the innate immune system [*5]

Introduction to the main receptors in innate immunity:

Dectin-1: Dendritic cell (DC)-associated C-type lectin-1 (DECTIN-1) is a novel CLR (C-type lectin receptor) expressed on myeloid cells and has been implicated in pattern recognition of pathogenic fungi [39] [40]. It binds fungal β-1,3 glucan-linked carbohydrates found in abundance in fungal cell walls [41] and has been shown to facilitate TLR2-mediated pro-inflammatory cytokine production by macrophages [42, 43] causing enhanced immunological response to infectious illnesses.

Stimulation of Dectin-1 causes activation of spleen tyrosine kinase (Syk) which in macrophages accounts for the induction of the respiratory burst, and in Dendritic cells for IL-2 and IL-10 release and phagocytosis[44-46]. Unlike other phagocytic receptors, Dectin-1 can also directly trigger cytokine production through an immunoreceptor tyrosine-based activation motif (ITAM)-like motif in its cytoplasmic tail.

Some studies propose that host-cell recognition of β-glucan is mediated mainly by dectin-1 [47, 48]. The studies on dectin-1's role have specially been cited in relation to fungal species such as *saccharomyces,*

candida, coccidioides, pneumocystis and *aspergillus* [42, 49-53]. Even though role of Dectin-1 in fungal immunity has been explored in good length, it requires further elaboration in other pathogens.

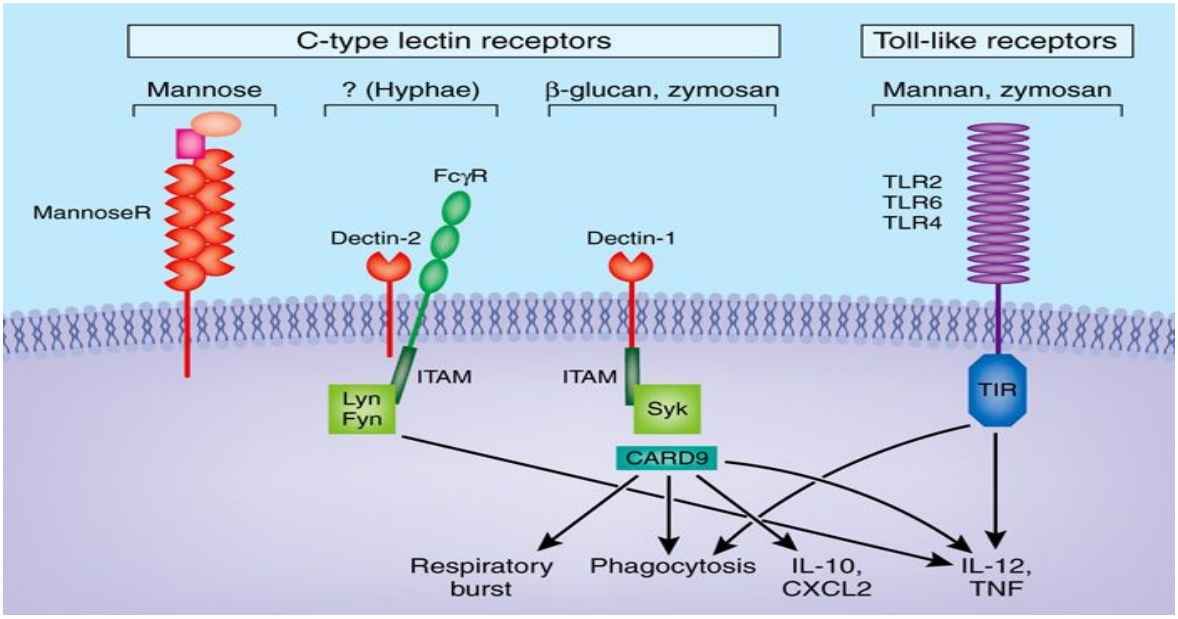

Fig. 10: Illustration of relation between Dectin-1 receptor, ITAM, Syk and CARD9 gene (Caspase recruitment domain family, member 9). The resultant effects (respiratory burst, phagocytosis, IL-10 production etc.) are also shown [*5]

Toll-Like Receptors (TLRs) have been referred to, in some studies, as a possible receptor for Dectin-1. They play a critical role in the early innate immune response to invading pathogens by sensing the presence of microorganisms. They have a similar DNA profile as Drosophila Toll gene and recognize highly conserved structural motifs expressed by microbial pathogens, the previously-mentioned pathogen-associated microbial patterns (PAMPs). PAMPs include various bacterial cell wall components such as lipopolysaccharide (LPS), peptidoglycan (PGN) and lipopeptides, as well as flagellin, bacterial DNA and viral double-stranded RNA. Stimulation of TLRs by PAMPs initiates signaling cascades that involves a number of proteins such as MyD88, TRIF and IRAK [54]. These signaling cascades cause activation of transcription factors AP-1, NF-κB and IRFs and induce the secretion of pro-inflammatory cytokines and effector cytokines (IL-12, TNF; see Fig. 10) that direct the adaptive immune response.

TLRs are located on the plasma membrane with the exception of TLR3, TLR7, TLR9 which are localized intracellularly [55]. Structurally, TLRs are transmembrane proteins with an extracellular leucine-rich domain and a cytoplasmic tail containing the Toll/IL-1 receptor (TIR) domain. TLRs are predominantly expressed in tissues performing an immune function, like spleen and peripheral blood leukocytes, and also tissues exposed

to external environment such as lung and the gastrointestinal tract, but the receptor subtype and pattern of expression varies among tissues and the cell types.

NOD-Like Receptors (NLRs) constitute a recently identified family of intracellular PRRs, which contains more than 20 members in mammals. Although the ligands and functions of many of these receptors are not known, their primary role is to recognize cytoplasmic PAMPs and endogenous danger signals. They are characterized by a tripartite-domain with a conserved nucleotide binding oligomerization domain (NOD) and leucine-rich repeats (LRRs). The general domain structure consists of C-terminal leucine-rich repeats involved in microbial sensing, a centrally located NOD domain and an N-terminal effector region made up of a protein-protein interaction domain such as the CARD, Pyrin or BIR domain.

The NOD-like receptors have been categorized into subfamilies on the basis of their effector domains: NODs, NALPs, CIITA(MHC Class II transactivator), IPAF, and NAIPs. NODs and IPAF contain CARD (caspase recruitment domain) effector domains, whereas NALPs and NAIPs contain pyrin (PYD) effector domains and three BIR (baculovirus inhibitor of apoptosis protein repeat) domains, respectively. They have only been cited in few studies in connection to possible β-glucan binding and uptake.

Among the noteworthy NOD-like receptors (NLRs) are NOD1 and NOD2, which are the first mammalian NLRs reported to sense intracellular microbial PAMPs, which contain one and two N-terminal CARD domains. They recognize peptidoglycan (PGN), an essential constituent of the bacterial cell wall. NOD1 and NOD2 detect specific motifs within the PGN. NOD1 senses the D-γ-glutamyl-meso-DAP dipeptide (iE-DAP) which is found in PGN of all Gram-negative and certain Gram-positive bacteria [56, 57] whereas NOD2 recognizes the muramyl dipeptide (MDP) structure found in almost all bacteria [58]. Thus NOD2 acts as a general sensor of PGN and NOD1 is involved in the recognition of a specific subset of bacteria. NALPs are a subfamily of NLRs that consists of 14 members characterized by the presence of PYD effector domains. Although precise functions of many NALPs are unknown, several have been reported to play a key role in the regulation of caspase-1 by forming a mutiprotein complex known as the 'inflammasome'. Caspase-1 participates in the processing and subsequent release of proinflammatory cytokines, such as IL-1β and IL-18 [59]. IPAF and NAIP5 constitute another set of NLRs. IPAF belongs to the CARD (caspase recruitment domain) subfamily whereas NAIP5 is a member of the BIR subfamily. Both have been shown to respond to flagellin, the main component of the bacterial flagellum, restricting the proliferation of intracellular bacteria such as *Salmonella typhimurium*, *Shigella flexneri* and *Legionella pneumophila* [60, 61].

DISCUSSION

RECEPTORS IMPLICATED IN β-GLUCAN RESEARCH:

Studies implicating Dectin-1 in β-glucan binding and uptake:

Various studies have been done to investigate the role of Dectin-1 in binding and uptake of β-glucans. Some of the latest studies have been elaborated in the following:

In 2002, a study was conducted by a Brown et al. [62] to define the contribution of various receptors to the recognition of β-glucans. They used Zymosan, a yeast-derived particle composed principally of polysaccharides β-glucan (the active component mediating the cellular effects), which has a number of desirable effects on immune function including the ability to confer resistance to tumors and various infections. This study focused on the ability of zymosan particles to stimulate cells of the reticuloendothelial system since it has a wide use in the investigation of many phagocyte responses. Using specific reagents, the study was able to define the receptors involved in the nonopsonic recognition of zymosan and soluble β-glucans in primary macrophages.

An antibody mAb-2A11, specific for Dectin-1, was generated by immunization of Fischer rats. It was first shown that nonopsonic zymosan recognition by primary macrophages was mediated by a β-glucan inhibitable receptor, which was different than Complement Receptor 3 (CR3). Then the role of Dectin-1 was examined in this process. It was observed that the antibody mAb-2A11 inhibited the binding of unopsonized zymosan, indicating that the mAb-2A11 (against Dectin-1) bound at or near the β-glucan binding site (Fig. 11).

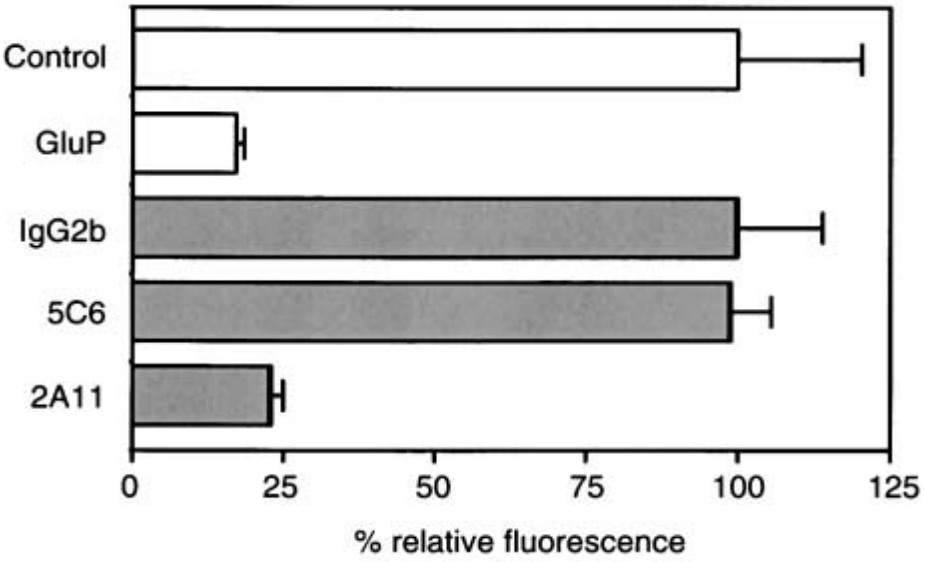

Figure 11. 2A11 (anti-Dectin-1), but not 5C6 (anti-CR3), specifically inhibits the recognition of unopsonized FITC-labeled zymosan by macrophages. Increased % of relative fluorescence indicates decreased binding and uptake of glucan particles. The level of inhibition with 2A11 is similar to glucan phosphate (GluP). ***Brown, G.D., et al. 2002; fig reproduced with permission***

The individual levels of inhibition by mAb-2A11 and 5C6 (the anti-CR3 antibody) depended on the degree of opsonization and the simultaneous addition of both antibodies, which has an synergistic effect. The study indicates that Dectin-1 mediates the β-glucan–dependent recognition of opsonized zymosan by macrophages.

Further studies were conducted in 2004 and 2005 on the role of Dectin-1 in the binding and uptake of β-glucans. Three noteworthy ones were from Reid et al., Adachi et al., and Willment et al.

Reid et al. [63] elaborated on the role of Dectin-1 as a PRR on macrophages (Ms), neutrophils, and dendritic cells (DCs). They study cited the mediation of the nonopsonic recognition of, and response to, soluble and particulate yeast ß-glucans by Dectin-1-carrying macrophages and bone marrow-derived DCs. Immunohistochemical detection of Dectin-1 was optimized and its expression was demonstrated on neutrophils, subpopulations of Ms in splenic red and white pulp, alveolar Ms, Kupffer cells, and Ms and DCs in the lamina propria of gut villi. This shows the consistency of Dectin-1's role in pathogen surveillance, the hallmark of which is the recognition of β-glucan component of microbial antigen.

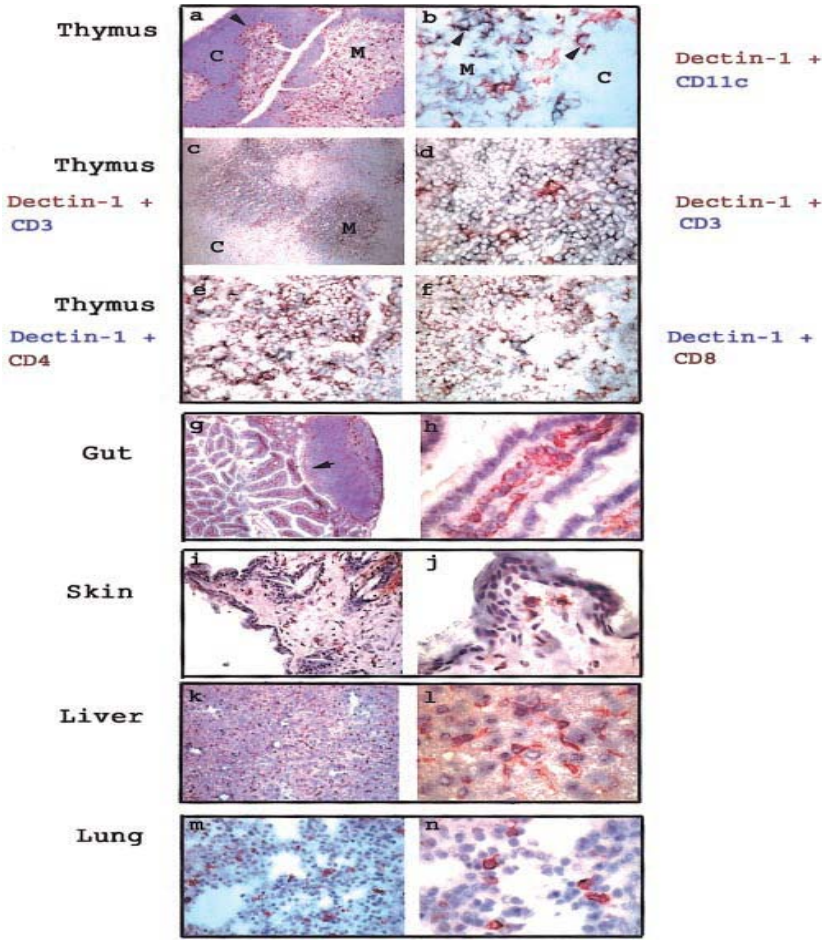

Fig. 12. Distribution of Dectin-1 in thymus & on other significant Macrophages & DC populations; *Reid et. al, 2004; fig reproduced with permission*

Dectin-1 expression was low on resident Ms and DCs of skin and was not detected on resident Ms or DCs in kidney, heart, brain, or eye. But the role of Dectin-1 as a coreceptor for T-cell activation was shown by its expression on DCs in the T cell areas of the spleen and lymph nodes. Strong expression of Dectin-1 on subpopulations of Ms and DCs in the medullary and corticomedullary regions of the thymus suggests a role distinct from pathogen recognition. Tissue localization thus revealed potential roles of Dectin-1 in leukocyte interactions during innate immune responses and T cell development.

Adachi et al. provided an apt characterization of β-glucan recognition site on Dectin-1 [64]. In their study, they tried to deduce the amino acid residues in dectin-1 responsible for β-glucan recognition. Using culture models of HEK293 cells transfected with mouse dectin 1 cDNA, they prepared 32 point mutants with mutations in the Carbohydrate Recognition Domain (CRD) and analyzed their binding to SPG (the Schizophyllan β-glucan). Out of these point mutations, the ones at Trp221 and His223 resulted in decreased binding to β-glucan as well as abscence of collaborative effect on TLR 2-mediated cellular activation in response to zymosan. This provided

further insight into the binding properties of β-glucan and Dectin-1, and showed the critical position of amino acid sequence W221-I222-H223 in the CRD of Dectin-1.

Willment et. al [65], following on Reid et. al's (2004) work, published a study identifying Dectin-1 as the major receptor for fungal β-glucans on murine macrophages. They demonstrated that Dectin-1 is widely expressed on all monocyte / macrophages, DC, neutrophils and eosinophils populations as well as on B cells and a subpopulation of T cells, indicating that human Dectin-1 is not myeloid restricted. Both functional β-glucan receptor isoforms GR-A & GR-B were expressed by the cells, with significantly higher GR-B expression on mature macrophages and immature DC, suggesting cell-specific control of isoform expression. They highlighted the insignificant modulation of Dectin-1 expression on macrophages during inflammation, using a novel "skin-window" technique. Overall, their study attested Dectin-1 as the major β-glucan receptor on immune cells contributing to the inflammatory response to the fungal carbohydrates. See Fig. 13.

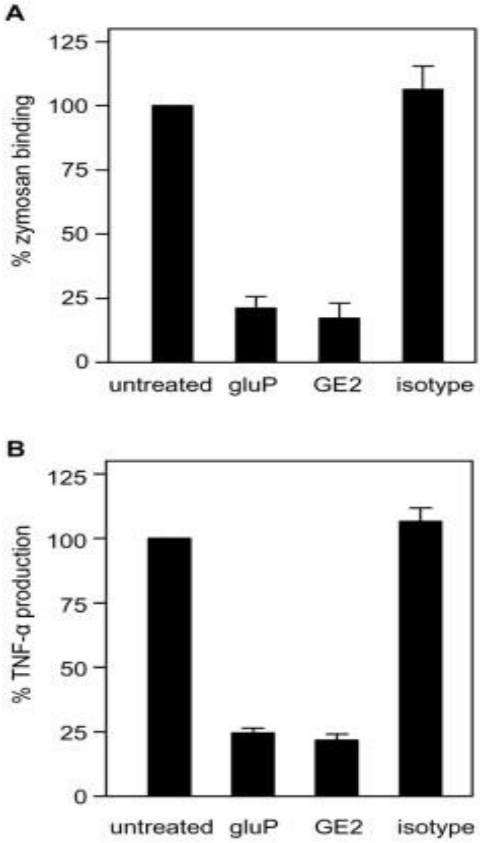

Fig. 13. Human Dectin-1: Zymosan binding (A) and TNF-α production (B), both are β-glucan-dependent, as demonstrated by inhibition with GluP (Glucan Phosphate) ***Willment et. al 2005; fig reproduced with permission***

In the Blood Journal in 2006, Yadav et. al published a study on the activation of macrophages by *Mycobacteria* species, which are known for causing highly prevalent diseases such as tuberculosis in developing countries. In this very important study, they implied that Dectin-1 functions together with TLR2 to mediate macrophage

activation by *mycobacterial* β-glucans [66]. In order to investigate whether Pattern recognition receptors (PRRs) work in concert to promote macrophage response to mycobacterial infection, they used bone marrow–derived macrophages isolated from mannose receptor (MR), CR3, MyD88, Toll-like receptor 4 (TLR4), and TLR2 knockout mice. With a series of experiments (Fig. 14) they were able to determine that mitogen-activated protein kinase (MAPK) activation, which is involved in a range of pro-inflammatory reaction cascades, and TNF-α production by macrophages is dependent on TLR2, but not TLR4, MR, or CR3. Especially, the TNF-α production by macrophages infected with *M smegmatis, M bovis* Bacillus Calmette-Guerin (BCG), *M phlei, M avium 2151-rough*, and *M tuberculosis H37Ra* required collaboration of the β-glucan receptor Dectin-1 with TLR2. Furthermore, Dectin-1 was noted to facilitate interleukin-6 (IL-6), RANTES (regulated on activation, normal T expressed and secreted), and granulocyte colony-stimulating factor (G-CSF) production by mycobacteria-infected macrophages, thereby establishing a significant role for Dectin-1, in cooperation with TLR2, to activate macrophage's proinflammatory response to a mycobacterial infection.

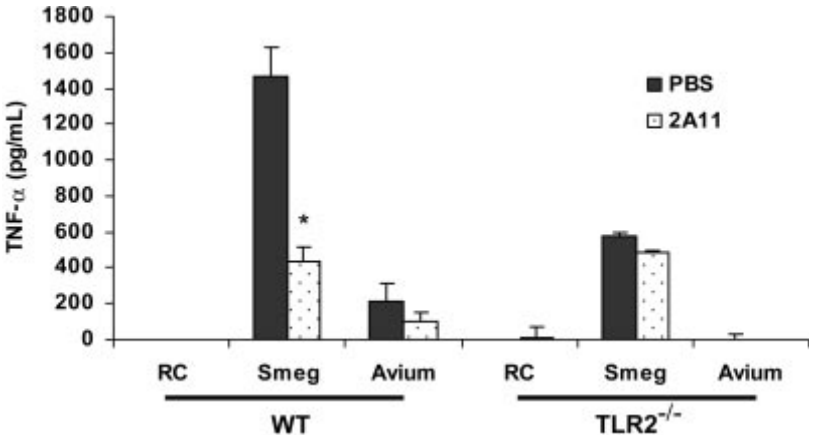

Figure 14. Dectin-1–mediated induction of TNFα by BMMs infected with *M smegmatis* requires TLR2. Notice the significant decrease in TNF production in TLR2 knockout mice. *Yadav et al., 2006; fig reproduced with permission*

In 2007, following the work of Talor et. al [67] and Dennehy et. al [68], Gow et al. [69] published a paper on further studies on the role of Dectin-1 in immune recognition of *Candida albicans* and other fungal species. While demonstrating that cytokine production by human and murine macrophages is dependent on the recognition of β-glucans by Dectin-1, they found that heat-killing of Candida species causes recognition of cell wall β-glucan by dectin-1, whereas live yeast cells interact mainly via mannan receptors. The study found that Syk-dependent and TLR-Myd88-dependent cytokine production from T-helper cells (Th2) and monocytes is related with Dectin-1, but the production of Interferon-gamma from Th1-type cells is rather independent of Dectin-1. Thus, the study concluded that in case of *C. albicans*, production of cytokines via monocytes or T-cells could be dependent or independent of Dectin-1 interaction with the cell wall β-glucans.

Among the studies from within the last two years, ones by Shah et al. (2008), Harada (2008) and Ujita (2009) et al. are of note.

Shah et al. [70] made an interesting discovery while seeking to determine whether Dectin-1 is present in microglia and whether it can mediate microglial responses to β-glucan. In their study, they reported, for the first time ever, that Dectin-1 is expressed in brain microglia as well as in the microglial cell line BV-2, where it serves as a receptor for β-glucan particles within a signaling pathway that involves Src family and Syk tyrosine kinases. They used unconjugated and PE-conjugated anti-Dectin-1, fluorescent labeled glucans as described by Ozment-Skelton et al. [71] and Rice et al. [29], and microglial cultures obtained from control and TLR2 knockout mice. β-glucan particles were shown to be phagocytosed by microglia in Dectin-1-dependent manner, and Dectin-1 was also required for subsequent Reactive Oxygen Species (ROS) production. However, unlike in immune phagocytic cells, binding of Dectin-1 by β-glucan did not cause any significant cytokine or chemokine production in microglia. But Zymosan binding on microglial receptors did lead to cytokine production, but that too in a Dectin-1 independent manner. It was postulated that cytokine production could well be TLR2 dependent in microglial tissue and that Dectin-1-mediated signaling in brain microglia could represent a new pathway of interest in neuroimmunology and neuroinflammation. See Fig. 15.

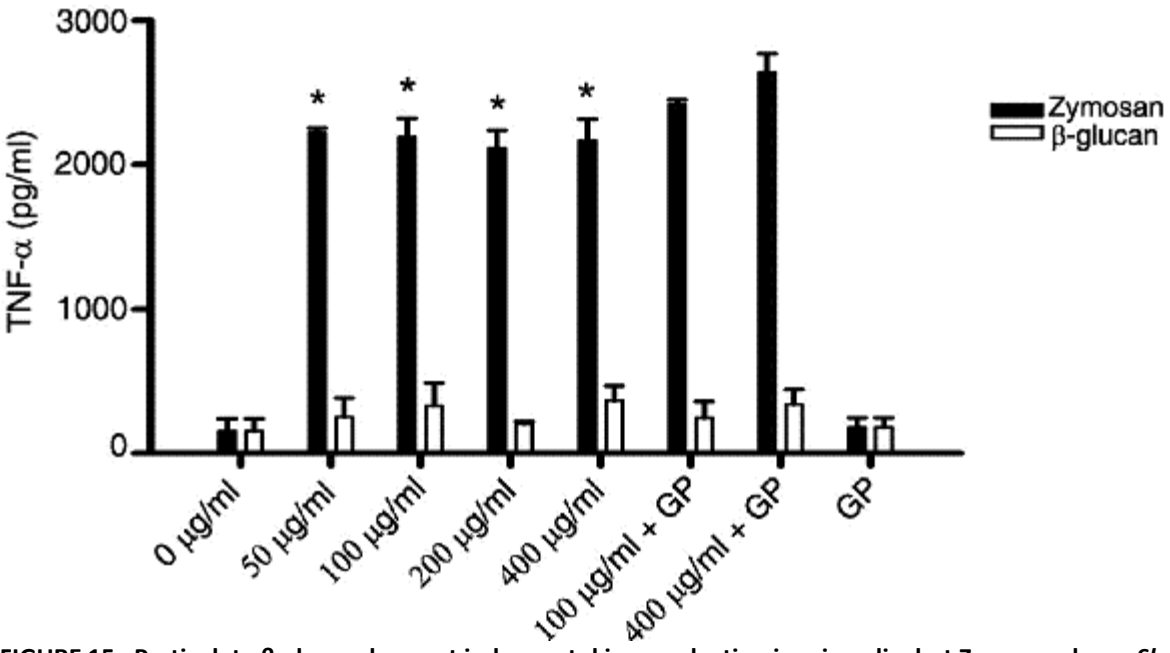

FIGURE 15. Particulate β-glucan does not induce cytokine production in microglia, but Zymosan does. ***Shah et al. 2008; fig reproduced with permission***

Harada et al., a group of researchers in Japan, published two papers in 2008 elaborating on the role of Dectin-1 in β-glucan binding and its subsequent immnomodulating effects in the bone marrow. One study is unique

in the sense that it describes Dectin-1 receptor on “dendritic cells” of the marrow. In the paper published in Journal of Interferon and Cytokine Research [72], they showed the result of their investigation on the effect of Sparassis crispa β-glucan (SCG) on bone marrow-derived dendritic cells (BMDCs) in DBA/2 mice. Having already proved earlier [73] that SCG has significant antitumor activity in DBA/1 and DBA/2 mice (inbred strains, with 98% genetic match), they went on to show increased CD80, MHC-I and MHC-II molecules' expression on the cell membrane of dendritic cells as well as increased interleukin-12p70, IL-6, and TNF-α production in mice of selected lineages. The magnitude of cytokine induction was higher in DBA/2 mice than in mice of other lineage, and so was the expression level of Dectin-1. Blocking Dectin-1 remarkably inhibited TNF-α production suggesting that the bone marrow DCs from DBA/2 mice are highly sensitive to the induction of cytokine production by the Sparassis β-glucan (SCG) *in vitro*, and that this sensitivity is related to the expression level of dectin-1. In the other paper published in the International Immunopharmacology journal [74], Harada et al. studied the contribution of dectin-1 and granulocyte macrophage-colony stimulating factor (GM-CSF) to immunomodulating actions of β-glucan. They elaborated on the strain difference in the reactivity of mice to SCG, showing that DBA/1 and DBA/2 are highly sensitive strains. Although complete abscence of Dectin-1 receptor would completely abolish the cytokine production in dendritic cells, as in the case of dectin-1 knockout mice, but controlling the level of endogenous GM-CSF alongside dectin-1 expression could regulate the reactivity to β-glucan. The point of focus was the role of GM-CSF, and the study's result indicates that GM-CSF production and dectin-1 expression are the key factors in the responsiveness to β-glucan.

In 2009, more research has been published [75, 76] with regards to the receptors for fungal β-glucans and their immune-stimulatory capability. But a stand-out study by Ujita et al. [77] sheds some light on the biological chemistry and carbohydrate binding specificity of human macrophage β-glucan receptor dectin-1. Upon expressing Dectin-1 as a fusion protein with an N-terminal hexahistidine tag and glutathione S-transferase (His-GST-hDectin-1) in an Escherichia coli cell-free translation system, the researchers assayed the recombinant protein for binding specificity with β-glucan in comparison with human dectin-1. The recombinant dectin-1 specifically bound to some β-glucans, but not to other carbohydrates, showing its relative specificity. The β-glucan binding of recombinant dectin-1 was inhibited by laminarin and laminarioligosaccharides, the soluble β-glucans, but not by other carbohydrates, again showing that dectin-1 is rather specific in its ligand-binding. Overall, the study shows that the recombinant human dectin-1, owing to its strict binding specificty, has potential use as a probe in identifying ligands in humans and tonic foods.

FUTURE PROSPECTS OF B-GLUCAN RECEPTOR AND CLINICAL STUDIES

Studying the response of human immune system to β-glucans extracted from various sources has been the mainstay of glucan studies uptil now. What we need to focus on is the exact nature of receptor studies and the intracellular pathways it activates.

Prospective research in production & purification of β-(1,3) glucans: The sources of natural occurrence, degree and character of polymerization, isolation protocols, degree of solubility of preparations… in fact all the trial-and-error phases of the preparation of β-glucan, especially the particulate one, point towards the fact that each isolated batch is heterogeneous. As extraction and purification processes drastically influence the glucan properties, numerous patents have been published on the subject. Processing may affect the molecular (chemical structure and DP), structural (molecular interactions) and functional properties (viscosity, water binding capacity and solubility), which may cause change in the physiological properties of glucans, and so can shearing damage due to mechanical processing or excessive heat treatment of food products. Decreased molecular weight and reduced viscosity can result from structural changes taking place during commercial purification [78]. Endogenous β-glucanases, if not deactivated through high temperatures, may cause increased depolymerisation of the β-glucans [79]. Even if in some cases the heterogeneity of β-glucan (different molecular size, branching, and crystalline or amorphous structure) does not principally change the *in vivo* activities, it still represents substantial complications for pharmacological research and regulatory authorities. Here, one could ask the question, how can we avoid such complications in preparing β-glucan supplements? The answer is "research".

Emphasis should be put on reliable research techniques to allow the problems of heterogeneity and nonexistent standards of various natural β-glucans to be solved. Improved isolation techniques could be the *first step in this regard*, so that products with closely defined chemical composition could be obtained and up-to-date physicochemical methods could be used for identification and analyses of these products. *Another method* could be to produce chemically-modified β-glucans, whose solubility could artificially be enhanced, but this may cause decrease in the glucan's biological activity. However, attempts have been made to construct semi-synthetic or synthetic probes suitable for immunological research. For example, in 2006, Descroix et al. proposed that short oligomers of glucose containing β(1→3) and β(1→6) linkages could be bound to a well-defined polymer carrier, so that such a "synthetic" β-glucan will interact with receptors of immunocompetent cells and elicit analogous reactions as natural β-glucan [80]. With the advent of synthetic β-glucan probes in immunopharmacological research, the shortcomings of natural glucans can be addressed.

Prospective areas of β-glucan research in Food industry:

Owing to its polysaccharide nature, β-(1,3) glucans have found a wide range of potential applications in food industry, like in edible films, feed for domestic animals and low calorie food.

There is some scope in the area of research in glucan gelling properties. Curdlan, a neutral gel-forming linear β-(1,3) glucan produced in *Agrobacterium* species, has been used as a biothickening and gelling agent in foods. Apart from being tasteless, colorless and odorless, studies have shown that heating process can produce different forms of curdlan gel with different textural qualities, physical stabilities and water-holding capacities. A prospective research area is to exploit these properties of Curdlan and other β-glucans to synthesize gels of differing strength for incorporation into gums for value-added food products.

An area of research for glucans in food industry is its application as an adjuvant for delivery of active compounds. Glucans can act as capsules or "tablet-like" ingredients which can deliver their content further, after ingestion. They are non-protein, heat-resistant capsule that could be filled with any product. A few experiments by Ono et al. (1996) and Shiomi et al. (2005) have already shown the promise offered by glucans in this regard.

When it comes to non-fat products, cereal β-glucan can be of use as soluble dietary fibre and as such can open avenues of research in food industry as stabilizer in low-fat products such as salad dressings, ice creams, yoghurts and cheese. This way, scientists could explore how β-glucans could help decrease the calorie content of food, as well as have beneficial effects on their gelation and rheological characteristics. In milk preparations, one could study how the gelling properties of β-glucan solutions could reduce curd cutting time and increases curd yields.

Prospective Medical and Pharmaceutical research areas:

Use of B-glucans in medical and pharmaceutical science is a potentially huge area of research. Cereal β-glucans have been studied to influence hyperglycemia and hypercholesterolemia, and the glucans obtained from bacteria, yeasts and fungi have shown promise as immunopotentiating and antitumorogenic agents. This paves way for research against serious infectious illnesses such as tuberculosis and AIDS.

β-glucan administration: With the goal of treating patients with β-glucans, parenteral (intravenous or intraperitoneal administrations) need to be investigated further. Since insoluble or hardly soluble β-glucans

cause significant adverse effects (granuloma formation, microembolization, inflammation, pain) when administered by parenteral routes [81], research needs to be focused on how oral administration could be optimized for quick delivery and higher bioavailability.

Hypercholesterolemia, Diabetes and Glycaemia: Cereal β-glucans have been studied for potential use as dietary fibre. Dietary fibre is a type of food substance mainly derived from plant material (cellulose, hemicellulose, pectin, lignin, etc) that resists human digestive enzymes. Research has shown that insoluble dietary fiber reduces bowel transit time [82], prevents constipation, reduces risk of colorectal cancer [83], and helps production of short chain fatty acids [84] for better handling of hyperlipidemia. And soluble dietary fibers, especially β-(1,3)(1,4) glucans, help lower blood cholesterol [85], sugar and insulin level [86], hypercholesterolemia [87], cardiovascular disease[88], cancer[89], and promotion of the growth of beneficial gut microflora (as a prebiotic). With this wide range of benefits already related to the use of β-glucan as dietary fiber, further studies are needed to elaborate on such clinical and preventive health benefits.

With special regard to hypercholesterolemia, the cholesterol-lowering potential of β-glucans is considered to result from effects manifest in the upper gastrointestinal tract via its ability to form a gel-like network and alter gastrointestinal viscosity. A few studies have implicated the amount and quality of fiber, increased intrinsic viscosity of the food in combination with fluids, maintenance of physical integrity of the food material and incomplete starch gelatinisation as the potential mechanism of action behind these effects [90] . It could be because intestinal viscosity reduces the cholesterol level by stimulating the production of bile acid. This could be via upregulation in the activity of cholesterol 7 α-hydroxylase (CYP7A1), an enzyme associated with the regulation of the pathway through which cholesterol is converted into bile acids. More research is required to elucidate the effect of β-glucan on enzyme and immune-regulation. Another potential area of cholesterol research could be to mix β-glucan with a non-digestible fat in one product for concomitant control of both high cholesterol and sugar level. Such mixtures, if developed through further research, could then be used as dietary supplement or as food additive when incorporated in products such as cereals, dairy products such as yoghurt or nutritional beverages, bakery products and prepared meals.

With regards to diabetes, β-glucan from different sources could be studied in a comparative fashion to highlight the different responses they elicit. For example, one could see if barley or oat β-glucans cause different level of reduction in blood glucose levels and if one of them is more effective in the regulation of glucose and insulin responses compared to the other. A study by Hallfrisch et al. published in the journal Cereal Chemistry in 2003 could be useful as a template where they investigated the use of β-glucans isolated

from barley and oats, and their corresponding effects upon plasma glucose and insulin responses in non-diabetic adults. But the extraction procedures, and factors such as dose, molecular weight and fine structure, and rheological characteristics of extracted and native β-glucans are important in this regard, as underscored by Wood et al. in 1994 [91].

An interesting prospective area in studying the medical and pharmaceutical effects of glucans is the effect of variation in viscosity of its preparations. One such study has already shown that the glycemic response of fiber-rich foods was inversely related to viscosity of the preparation [92]. Another group of researchers (Tappy et al.) also found that inclusion of oat β-glucan into breakfast cereals could reduce the postprandial glycaemic response by up to 50% [93]. Further studies could be done on the combination of altered viscosity and modified structure of β-glucans, and noting its resultant neutraceutical effects.

Inclusion of β-glucan could be made to a staple or regular-use food and could be studied over longer periods. For example, Yokoyama et al. (1997) and Knuckles et al. (1997) have done comparisons of blood glucose and insulin responses of healthy individuals following the ingestion of different sources of β-glucans. This way, the ability of β-glucans to influence the rate of starch degradation and hence the glycaemic index of foods could be studied with regard to obesity and diabetes, and standards could be set with blood glucose levels of diabetic and pre-diabetic individuals with the use of β-glucan rich foods.

Prospective research as Immunopotentiator: β-glucan could have important application as an immunopotentiator. Through earlier research, a wide variety of mushroom polysaccharides including scleroglucan, lentinan, schizophyllan, and grifolan have been described as biological-response modifiers [94] acting through mechanisms mediated by the immune system, including its nonspecific stimulation. Such polysaccharides obtained from bacterial, fungal or yeast sources have a beneficial effect on a variety of infectious or cancerous disease processes. In this regard, the baker's yeast cell wall preparation (Zymosan) has been a milestone. Being the first defined pharmaceutical yeast product with immunostimulatory activity, its active component was identified to be β-d-glucan and was reported to stimulate phagocytosis, cytotoxic activity in macrophages, and other biological activities [95]. Further research could be done on the mechanism behind these actions, especially the receptor studies of the innate immune system. One could investigate the signaling cascades which regulate gene expression concerned with the secretion of co-stimulatory molecules and increased antigen-presentation activity which help direct the adaptive immune response.

Research in anti-tumorigenic effects: Although, anticarcinogenic effect of many mushroom polysaccharides such as lentinan, grifolan, scleroglucan and schizophyllan have been described in earlier studies, the mechanism of their anti-tumour action is still not completely clear. It remains to be seen whether these β-glucans appear to mediate their antitumour activity by activation or augmentation of the host's immune system, via activation of leukocytes and production of inflammatory cytokines [96], or may be a combination of all of these. Although glucan-specific receptors have been found on phagocytic cell membranes and their role in phagocytosis and killing has been described, there tends to be a greater variation in activity against various cancer types, such as sarcomas, breast cancer, adenocarcinoma, colon cancer and some leukemias. To normalize this degree of variation, and to define the exact mechanism of anti-tumorigenic effect of β-glucans, further research is needed.

Anti-HIV & AIDS effects: The HIV has the ability to incorporate its DNA, multiply inside, and cause destruction of the T-helper cell subtype of lymphocytes, while dodging the normal scavenging leukocytes such as macrophages. In the past, extracts of *Lentinus edodes* (lentinan) has been found to stimulate specific T-helper cells in healthy humans while stimulating lymphokine activated killer (LAK) activity in combination with Interleukin-2[97]. Recent development concerning the direct utilization of β-glucans for treating AIDS are rare, but there have been proposals to establish clinical trials with *Grifola frondosa* extract, sulfated β-glucans, curdlan and lentinan sulfate or in combination with more conventional drugs [98]. Further research could be done in this regard to prove the efficacy of β-glucan preparations in treatment of AIDS.

Potential Prebiotic Applications: Prebiotics are compounds that help in sustenance of beneficial microorganisms in the intestinal tract. These microorganisms help in lactose digestion, cholesterol reduction, antimicrobial effects, immune system stimulation etc. and include *Lactobacilli, streptococcus salivarus* and *Bifidobacteria* species in particular. Oat β-glucan has been reported to selectively support the growth of *Lactobacilli* and *Bifidobactera* in rat experiments and in *in vitro* studies [99]. Further research could be done based on these observations to develop several patents on the use of β-glucans (or their corresponding hydrolysates) as prebiotics.

Other Therapeutic Applications: Many other therapeutic applications could be studied for β-glucans. One such utilization could be as radioprotective agent. This has already been put into perspective by an earlier study showing that β-glucan is able to protect macrophages from free radical attack during and after the radiation allowing these cells to continue to function in the irradiated body [100]. Another prospective area is to study glucan as a DNA-transport tool. β-glucan has shown the ability to function as a gene carrier with its

hydrogen-bonding triple-helix polymer structure (as in schizophyllan, curdlan, lentinan, scleroglucan) binding to a nucleic acid. The resulting nucleic acid-polymer complex can be used as a vector with further benefit as nucleic acid-protecting agent as it is resistant to nuclease. Further studies could establish this usage more clearly.

It will be interesting to study β-glucans for the treatment and prevention of digestion troubles such as constipation, inflammatory bowel disease, or stomach troubles using earlier studies as a template. More potential uses of glucans have been proposed for arthrosis, dermatitis and athlete's foot and oral cavity infections, and could be studied in detail with further research.

Prospective research in prevention of side-effects of β-glucan use: While there are predominantly positive pharmacological effects of β-glucans, the unfavorable side effects of glucans cannot be overlooked. Therefore, a theme for future research could be prevention of hazardous or side effects with β-glucan use. Such effects may arise from independent use of glucans, but are more likely to occur with the intake of glucans as an adjuvant.,.

Intramuscular administration of β-glucan is painful, and induces an inflammatory reaction and granuloma formation at the puncture site. β-glucan may cause an inflammatory reaction itself, so that a naturally occurring "physiological" inflammation in response to a noxious impact might become compounded and "pathological" with β-glucan's presence. As Tanriverdi et al. described in 2005, the resulting insult and tissue damage may herald the development of immunopathological (e.g., autoimmune) processes. Even worse, a generalized inflammatory process may develop into shock and multiple organ dysfunction syndrome (MODS). A future research area could be the development of preventive or therapeutic strategies in such case scenarios.

Nitric oxide is produced in macrophages by inducible nitric oxide synthase (iNOS). It has cytotoxic effect upon tumor cells [101] and shows distinct impact on many pathogens. iNOS synthesis (are you sure that you do not mean nitric oxide synthesis instead of iNOS synthesis ?) is triggered by binding of β-glucan to a PRR on the macrophage surface. Besides tumoricidal and bactericidal effect, nitric oxide can also damage tissues and DNA and high iNOS concentrations can cause septic shock. The sustained action of the activating pathogen or tumor cell causes continuous expression of iNOS and higher amount of nitric oxide and causes vasodilatation of veins. A group of researchers studied this phenomenon [102] and concluded that the drop in blood

pressure may come to a dangerously low level. A prospective area of research could be to study the risk of this eventuality with the use of adjuvant β-glucan therapy in infectious diseases or cancer.

The Syndrome Of Toxic Organic Dust (STOD) is defined as the aftermath of inhalation of intact cells, fungal or yeast bodies, home, agricultural or industrial dusts [103]. The condition is characterized by respiratory tract reactions that include pneumonia, cough, chronic bronchitis, rhinitis, headache, and irritation of the eyes and throat [104]. It has been found that the cause of these complaints is β-glucan, which, through activation of macrophages, monocytes, and leukocytes, causes increased secretion of inflammatory components (i.e., TNFα and IL-8). Research emphasis could be put on the tools to modulate such (over)activation of phagocytes against cell-wall β-glucans.

Drug interaction is a widely-studied phenomenon in modern medicine. With the use of β-glucans (pro-inflammatory compounds), there is a risk of competitive drug interaction with anti-inflammatory drugs. A study of reference in this regard [105] demonstrated the lethal toxicity elicited by a combination of β-glucan and Indomethacin (a nonsteroidal anti-inflammatory drug) in experimental mice. It has been proposed that simultaneous use of both agents may cause cytokine level derangement and Systemic Inflammation Response Syndrome (SIRS). A future research area could be to study how such an interaction could be prevented or controlled.

THE GLUCAN GROUP IN TROMSØ:

The Glucan Group in Tromsø has been in function for a couple of years now. The group focuses on four different areas of β-glucan research.

One subgroup has used immunhistochemistry to study molecular marker-potential of Dectin-1, scavenger receptor, TLR2, MR, MyD88 and CR3 in the human gastrointestinal tract. These receptors have been described to play a role in binding and uptake of glucans and the subgroup is working to elicit this mechanism in the distal small intestine (ileum).

One group worker has been assaying the biological activity of different batches of both soluble and non-soluble β-glucan produced by Biotec Pharma, AS. In the last year, focus has remained on using the mouse macrophage cell line RAW transfected with the Dectin-1 gene as a tool to analyse the β-glucan response by measuring cytokines in the conditioned medium by ELISA.

My focus as Masters student has shifted from cell-line studies to mouse intestinal specimen studies. I have worked with instestinal (ileal) explants obtained from mice. Small pieces (3-4 cm) of ileum tissue were cut loose and thoroughly wased. Thereafter they were incubated with FITC-labeled glucan in culture medium for a few hours, and before fixation. The protocol has been devised for both FITC-SBG and the FITC-NBG and we have been trying to overcome the difficulty of inconsistent tissue morphology, which is a drawback in identifying the cells. More time and effort is needed to establish this method accurately.

Two group members have worked with human peritoneal macrophages from healthy patients and have noted active uptake of FITC-SBG and FITC-NBG. There is a degree of variation found in the uptake activity of these cells, ranging between 10-90% within the different macrophage batches for FITC-SBG uptake. Interest remains in noting if single cells are able to take up both SBG and NBG, and if different uptake-mechanisms are involved. Three main uptake-mechanisms are studied: Clathrin-mediated endocytosis, macropinocytosis/phagocytosis, and lipid raft/caveola-mediated endocytosis. The subgroup aims to define relative prevalence and specificity of binding and uptake of a ligand with these different methods.

Preliminary studies in culture of cells from rat liver has shown that non of the the scavenging sinusoidale endothelial nor the Kupffer cells did take up FITC-SBG, whereas a few unindetified liver cells were able to accumulate the FITC-NBG. Further, by using both human macrophages and monocytt-derived DC, the group aims to define other lectins which are involved with binding and uptake of β-glucans, such as theMR, DEC-205, DC-SIGN, Dectin-2 and Scavenger Receptor C-type Lectin.

Also, this subgroup has established a protocol of isolation of monocytes from human blood. Monocytes are then cultured *in vitro* in culture dishes with IL-4 and GM-CSF and by using a cocktail that includes IL-1, TNF-α, IL-6 and PEG_2, the monocytes differentiate into DC. The sub-group further aims to study and analyze the effect of SBG and NBG on these cells.

One group member has been working with analyzing macrophage-RNA from both mouse and human specimen after stimulation with SBG. She published a study in this regard in 2007 [106], with more coming in due course of time.

Another group member has been investigating the effect of SBG and NBG glucan on myocardial infarcts in experimental pigs before and after the artificially-induced infarction. At the same time, NBG has been used in patients prior to heart surgery and its influence in the disease prognosis is observed.

CONCLUSION

Among many known and tested immunomodulators, polysaccharides isolated from various natural sources occupy a prominent position. An important group of these polysaccharides is represented by the homopolymers of β-glucose, called β-glucans. Due to their very low-to-negligible toxicity, they have tremendous potential use in a variety of diseases, such as infections, irradiation diseases, and foremost on neoplastic growth. Branded earlier as a food additive and an "alternative" remedy in its early development days, the wave of enthusiasm about β-glucans declined, the main reasons being insufficiently defined preparations and non-specific and/or complex effects. But over the last decade or so, modern medical research has reached a phase where the basic mechanisms of glucan effects are known and the relationship between structure and activity has been outlined rather clearly. It seems now that β-glucans will finally take the position they deserve in diagnostic and preventive medicine.

Being widely distributed within microorganisms in which they act as membrane components, and with no biosynthesis in mammals, β-glucans have been observed to activate the immune system of their host. Since the discovery of their involvement as immunomodulating agent, numerous studies have been published dealing with purification procedures, analytical chemistry, synthetic processes, chemical modification, physicochemical properties, and assessment of the biological and medicinal effects of the natural polysaccharides through *in vitro* and *in vivo* studies. Studies should be focused more on discovery of the receptors present on immunocompetent cells and scope and limitations of chemical synthesis and modifications of β-glucans.

As the research on the properties of new plant or microbial polysaccharides continues to grow, we need to streamline the commercialization of such compounds against traditional products, and somehow balance the improvement in original structures with the cost of production and development. As it looks at this point, β-glucans will have significant development in the next few years, notably in non-food sectors, with the most promise seemingly possible in therapeutic applications. Such applications include immunomodulators, antitumorogenic, antiviral (AIDS), anti-hypercholesterolemic and anti-hyperglycemic agents. Moreover, specific action as food additives could be ascribed and research with β-glucans. Ultimately, the pharmaceutical industry may provide new markets for chemically-modified glucans, like the sulfated ones, and help develop new generations of polysaccharides with more beneficial biological activities.

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