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Update on the Immunomodulating Activities of Glucans

Nicholas R. Di Luzio

Department of Physiology, Tulane University, School of Medicine, New Orleans, LA 70112, USA

Introduction

This report will accent studies which have been published within the past two years relative to the immunopharmacology of glucan. The limitation relative of this coverage of glucans is based upon the fact that a number of reviews have recently appeared [5, 8, 10, 11, 18, 29]. The proliferation of review articles demonstrates the growing interest in these unique immunomodulators. Di Luzio [11] presented an overview of glucan as a broad spectrum enhancer of host defense mechanisms against bacterial, viral, fungal, parasitic infections, and neoplasia, as well as the role of glucan as a unique adjuvant in diverse vaccines. The role of glucan as an inducer of hemopoiesis in normal as well as sublethally and lethally irradiated animals was also denoted. Additionally, publications pertaining to the unique feature of β-1,3 glucans, which to the knowledge of the author is not displayed by any other drug, whereby certain infectious diseases can be modified in invertebrates and plants were cited. In an extension of these comparative immunobiologic observations, glucan has been recently reported to enhance hypersensitivity reaction in potatoes induced by arachidonic acid derivatives [34]. Enhanced delayed hypersensitivity reactions have been observed in glucan-treated experimental animals [1, 24].

Browder [5] discussed the role of glucan immunomodulation in surgical infections. Cook et al. [10] considered the immunomodulation of protozoan diseases employing glucan as an adjuvant. Patchen [29] critiqued the comparative aspects of a variety of glucans and glycans on hemopoietic activity, as well as on bone marrow and splenic hemopoietic recovery in mice which received 650 rads of total body cobalt radiation. Jacques [18] reviewed the differential properties of glycans prepared from (a) *Rhodotorula rubra*, (b) *Aerobacter levanicum*, (c)

Offprint requests to: Nicholas R. Di Luzio, Department of Physiology, Tulane Medical School, 1430 Tulane Avenue, New Orleans, LA 70112, USA

Schizophyllum commune, (d) *Lentinus edodes*, and (e) *Saccharomyces cerevisiae*. The comparative biologic properties of these glucans and glycans were considered. Jacques comparative observations [18] were extended by the recent review on "Immunopharmacology of lentinan and the glucans" by Chihara [8]. In view of these diverse reviews, this report will be restricted to an update on glucans.

Historical Background Relative to the Development of Glucans

One of the questions usually posed relates to how the immunomodulator activities of glucan were discovered [38]. In the early 50s our laboratory attempted to define the role of macrophages in the metabolism of chylomicrons and cholesterol, and their possible role in the development of coronary artery disease. At that time the technique employed to evaluate the physiology of macrophages was to attempt to depress macrophages and ascertain the consequence using colloidal carbon or trypan blue to produce so-called "reticuloendothelial blockade." Extensive studies in our laboratory have demonstrated that while, from an anatomic and histologic point of view, the accumulation of dyes and particulates in macrophage elements might lead to the assumption that these cells are functionally impaired, functional phagocytic data indicated marked activation of these cells. Thus, the technique of "reticuloendothelial blockade" was found to be extremely inappropriate in the evaluation of the contribution of macrophages to homeostasis. We initially undertook an evaluation of a variety of pharmacologic agents which might possess the ability to either enhance or suppress macrophage function; however, these drugs initially employed were minimally effective. In 1957, Benacerraf and Sebestyn [4] presented a report on the influence of intravenously administered zymosan, a crude *S. cerevisiae* cell wall fraction, on macrophage activation. One of the findings of this study was the stimulation of macrophages, as reflected by phagocytic activity, as well as the development of hepatosplenomegaly [4]. Employing this initial observation, our laboratory decided to isolate, if possible, the active component in zymosan which related to its ability to enhance macrophage function as well as induce proliferation of macrophage elements in liver, lung, and spleen. The results of our study, conducted by Dr. Riggi and published in 1961, indicated the active fraction of zymosan was glucan [38]. This study initiated the era of glucan research. Retrospectively the results of glucan research over a quarter of a century has exceeded our initial anticipations.

Chihara et al. in 1969 [9] subsequently isolated a β-1,6; β-1,3 glucan from *L. edodes* designated lentinan and demonstrated its influence on the inhibition of sarcoma 180 in allogeneic mice. Since that time a variety of glucans have been prepared from an extensive array of single and multicellular fungi (Table 1) and evaluated as to their ability to enhance host resistance to either infections or malignancies. In general, the most effective immunomodulating polysaccharides of the glucan class have been derived from *S. cerevisiae*, *L. edodes*, and *S. commune* (Table 2). This study will concentrate on those glucans which are chemically pure and predominantly beta-1,3 polyglucose structures.

Table 1. Sources of glucan employed in experimental studies

A. auricula-judae
A. faecalis
S. polytricha
C. latifalium
C. purpurea
C. vagum
C. versicolor
C. utilis
L. edodes
P. cocas
P. fermentans
P. occidentalia
P. umbellatus
S. cerevisiae
S. commune
S. glucanicum
S. salivarius
S. sanguinolentum
W. robertsii

Table 2. Polyglucoses currently under clinical evaluation

Compound	Source	Structure
Glucan		
Particulate		
Soluble (Dirocan)	*S. cerevisiae*	β-1,3, helical (triple)
Krestin (PSK)	*C. versicolor*	β-1,4; β-1,3; β-1,6, protein complex
Lentinan	*L. edodes*	β-1,6; β-1,3, helical
Schizophyllan (SPG)	*S. commune*	β-1,3; β-1,6, helical (triple)

Influence of Glucans on Bone Marrow Cells and Recovery From Radiation Injury

One of the major consequences of macrophage activation with yeast glucan has been the pronounced elevation of colony stimulating activity in serum [8, 29–33, 42]. In an extension of these studies, Satoh et al. [42] have reported that a single intraperitoneal injection of PSK, like yeast glucan [29], resulted in significant elevation of colony stimulating factors (CSF). The increase of colony stimulating activity was dose dependent. Doses of 62.5 and 125 mg/kg of PSK produced no increase in colony stimulating activity, however, a dose of 250–1000 mg/kg was associated with a rapid increase in colony stimulating activity followed by a rapid decline. Yeast glucan has been demonstrated to be very effective in enhancing CSF at doses where PSK is ineffective [30]. The intraperitoneal administration of PSK was observed to induce two active CSF fractions on the the basis of ion-exchange chromatography. Both isolated fractions had the capability of enhancing hemopoietic colonies in culture.

Satoh et al. [42] suggested that the elevation in colony stimulating activity may be one of the tumor defense mechanisms in vivo whereby host stem cells are induced to proliferate, thus increasing the total available pool. The enhancement of all cellular elements of blood, particularly monocytes, neutrophils, and lymphocytes would increase host defense mechanisms and assist in maintaining the sterility and purity of the internal environment against a variety of noxious agents.

Patchen and MacVittie [30] continued their outstanding studies on the influence of yeast glucan and other glycans on pluripotent stem cells and myeloid and erythroid progenitor cells. They reported that in a study of six different doses of glucan ranging from 0.1–2.0 mg administered to C3H/HeN mice produced a dose-related increase in all aspects of hemopoiesis, i. e., pluripotent hemopoietic stem cells (CFU-s), granulocyte-macrophage (GM-CFC), pure macrophage (M-CFC), and erythroid (BFU-e, CFU-e) progenitor cells. In bone marrow CFU-s content increased while CFU-e and BFU-e content decreased with increasing glucan doses. Elevated levels of granulocyte-macrophage colony stimulating activity (GM-CSA) have been found in serum, peritoneal fluid, and cells obtained from lung, spleen, and the peritoneal cavity of particulate glucan-injected mice.

Patchen et al. [31] also studied the influence of six different soluble glucans and two soluble mannans as well as fructan as to their ability to enhance hemopoietic recovery in C3H/HeN mice when administered one hour before or one hour after a 6.5-Gy dose of cobalt-60 radiation. Hemopoietic recovery was assessed by the endogenous spleen colony assay. Based upon their previous work [29] particulate yeast glucan was employed as a positive control. Patchen et al. [31] observed that mannan and two soluble yeast glucans designated glucan-F and glucan-S, enhanced endogenous colony formation four to five-fold in a manner equivalent to that of the positive control, i. e., particulate glucan. Levan and SPG (Schizophyllan) produced a smaller increase of approximately 2.7-fold. In contrast, lentinan, krestin, mannan, and another soluble yeast glucan fraction designed glucan-C did not enhance hemopoietic recovery above radiation control values. It is obvious that the variety of glucans which are presently being investigated do not possess equivalent or even similar biologic activities when the immunologic profile of these agents are evaluated [see also 8].

In studies conducted by Patchen et al. [31] when the agents were administered 1 hour after radiation exposure, endogenous colony formation was also enhanced by mannan, schizophyllan, glucan-S, krestin, and glucan-F. Again mannan, levan, lentinan, and glucan-C did not possess activity. These studies clearly indicate the possibility of enhancing recovery from sublethal and possibly lethal radiation in the hemopoietic range of radiation injury by the employment of glucans. Indeed, Patchen [29] reported that particulate glucan administered to mice 24 hours before 900 rads cobalt-60 radiation induced an approximate 45% survival in contrast to 0% in the control group. Pospisil et al. [33] have demonstrated that a single pre- or post-irradiation administration of β-1, 3 glucan enhances hemopoietic recovery in sublethally gamma-irradiated mice. Pospisil et al. [33] also reported that pretreatment of mice with glucan, isolated from *S. cerevisiae*, prior to radiation injury significantly reduced the lethal effects of radiation. Postirradiation treatment was less effective.

Macrophages clearly have a unique role in the control of stem cell proliferation and hemopoiesis [29]. The ability of glucan to enhance macrophage secretion of colony stimulating factor allows a definitive and unique control of the dynamics and kinetics of bone marrow. These studies support the observations of Di Luzio and Williams [12] that the administration of particulate glucan to animals which receive cyclophosphamide at doses which induce profound leukopenia, was able to counteract the development of leukopenia as well as decrease the susceptibility to *S. aureus* infection.

Patchen et al. [32] have recently reported that the administration of glucan-F, a soluble yeast glucan prepared by our laboratory, either prior to or one hour after 650 radiation exposure of mice resulted in more rapid recovery of pluripotent stem cells as well as committed granulocyte, macrophage, and erythroid progenitor cells. Patchen et al. [32] suggested that this observation may explain the mechanism by which yeast glucans possess the ability to enhance survival of a significant number of animals (approximately 60%) that received an otherwise lethal radiation dose. It is obvious that this area of glucan research will be one of significant future interest.

Role of Glucans in Modification of Infections in Control and Immunosuppressed States

In an extension of studies relative to the ability of β-1,3 glucans to enhance resistance to infectious agents in experimental animals, Browder et al. [7] conducted studies in mice that underwent midline laparotomy as a surgical stress. The animals were subsequently challenged intravenously with 3×10^6 *C. albicans*. The detrimental effect of the surgical procedure on survival following *C. albicans* infection was manifested by a 20% survival in the surgery *C. albicans* infected group, in contrast to a 47% survival in the control non-surgery infected group. Protection against *C. albicans* was observed in the glucan non-operative but anesthetized mice as well as the operated group. The glucan-treated non-operated mice manifested 100% survival following *C. albicans* infection, while the surgical stressed-infected group had a 73% survival. This 73% survival in the glucan group is in marked contrast to the 20% survival in the surgery-infected non-glucan-treated group. Histopathologic studies that were conducted in these animals indicated that glucan markedly inhibited the renal pathology and renal candidiasis which was associated with *C. albicans* infection in both the control and surgical-stressed group. The authors concluded that glucans may be effectively employed at a future date in patients that are at risk for developing postoperative infections.

Browder et al. [6] have also recently reported the protective effect of nonspecific glucan immunostimulation in postsplenectomy sepsis. This study was undertaken since it has been amply demonstrated that there is an enhanced risk of both children and adults to develop severe sepsis following splenectomy. Prophylactic antibiotics and bacterial vaccines have been utilized, with limited success, in an attempt to inhibit the unusually high degree of morbidity and mortality which develops in splenectomized individuals. In an effort to evaluate whether glucan could be employed as an nonspecific immunostimulant to modify postsplenectomy pneumococcal sepsis, studies were conducted in mice that were intranasally-treated with

1×10^9 *Streptococcus pneumonia.* Both sham operated and splenectomized animals were employed both in the presence and absence of glucan administration. It was observed that glucan administration significantly increased survival in the splenectomized infected group (75%) compared to the 27% survival in controls. Browder et al. [6] concluded that nonspecific immunostimulation may have significant potential as a treatment strategy against postsplenectomy infections.

Kimura et al. [21] reported that macrophages from particulate glucan-treated animals had significantly increased rates of phagocytosis and killing of *Staphylococcus aureus.* Increased phagocytosis and killing was also observed when *Klebsiella pneumoniae* was employed. In contrast to the beneficial effects of glucan administration on *S. aureus* infection and *Klebsiella pneumoniae,* only transient protection was observed with group C *streptococci.* Histologically the rats that received glucan showed a greatly increased number of pulmonary macrophages. The authors suggested that yeast glucan significantly enhanced intrapulmonary bacterial host defense mechanisms resulting in increased intrapulmonary killing of bacteria.

Sakamoto et al. [40] have recently reported that lentinan administration will also increase resistance of rats to *Listeria monocytogenes* infection. The increased resistance to infection was associated with an elevation in C3. This complement elevation occurred even in a malnourished state, i. e., when rats were maintained on a 3% and 0.5% protein diet. Sakamoto et al. [40] observed that malnourished animals, as well as controls, which received lentinan displayed a 100% survival against a 5×10^6 Listeria challenge in contrast to 20% survival in the control groups.

The importance of complement in the mediation of the increased resistance induced by lentinan to infection was suggested by the observations that the administration of cobra venom factor resulted in a 14% survival in the control group following administration of 2.5×10^7 Listeria. In contrast lentinan-treated mice which had elevated C3 concentrations showed a 100% survival. These studies again stress the importance of macrophage secretory function, i. e., production of various complement components as well as macrophage number in the protection of the host against bacterial diseases.

Anti-Neoplastic Activity of Glucans

In the evaluation of the immunotherapeutic effects of glucans as well as other biologic response modifiers, an important consideration in the potential clinical use of these drugs is the appropriateness of animal models. Recently Di Luzio et al. [13] undertook a study to evaluate the effects of glucan on tumor growth in conditions of genetically diverse host-tumor relationships. It was observed that in allogeneic mice, glucan treatment resulted in profound inhibition (95%) of the growth of adenocarcinoma. Tumor incidence which was 100% in control groups, ranged from 33–50% in the glucan-treated group during a 15-day study period. In contrast in syngeneic mice, the inhibition of tumor growth ranged from 34–64%. In an autochthonous tumor model employing aging AKR mice which developed spontaneous leukemia in mid-life, it was observed that the biweekly administration of particulate yeast glucan for a six month period resulted in significant pro-

longation in survival. Indeed, those animals autopsied at the end of this period showed no evidence of malignancy.

The importance of macrophage elements in mediating host resistance to malignancy was manifested in this study when methyl palmitate, a macrophage suppressant compound was employed [13]. In the spontaneous AKR tumor model, the biweekly administration of methyl palmitate decreased survival compared to the controls as well as, of course, glucan-treated animals. In general, the administration of methyl palmitate to allogeneic and syngeneic mice bearing adenocarcinoma tumors resulted in increased incidence and growth of the tumor. It was concluded that tumor growth and development appeared to be generally inversely related to macrophage functional status, i. e., if macrophage function is increased, tumor growth is decreased, while if macrophage function is decreased, tumor growth is enhanced. The observations that particulate glucan suppressed natural killer (NK) cell activity [22] while mediating increased host resistance to malignancy certainly eliminates NK cells as a factor in glucan-induced resistance to neoplasia as well as infectious diseases.

Suga et al. [43] also studied the antitumor effect of lentinan in syngeneic and autochthonous murine hosts and the influence of lentinan on chemically induced carcinogenesis. In methylcholanthrene-induced carcinogenesis it was observed that lentinan had a significant effect when DBA and SWM mice were employed, but had no effect when C57BL/6, C3H/He, and BALB/c mice were utilized. The timing of lentinan administration was also a factor in the anti-tumor response. When lentinan was given daily for 10 days three weeks after the administration of methylcholanthrene, effectiveness was observed. It was less effective when administered six weeks after methylcholanthrene administration. No correlation was observed in this study relative to natural killer cell activity or phagocytic macrophage function in regards to lentinan action [43]. Employing the sarcoma 180 model, it was found that the tumor inhibitory effect of lentinan was most effective in DBA/2, SWM/Ms, and A/J mice, the latter being deficient in NK cell function. These findings against stress that the models employed are critical in the evaluation of biological response modifiers and that NK cells appear not to be important in the anti-tumor activities of glucans.

Rose et al. [39] also studied the influence of lentinan against Lewis and Madison 109 lung carcinomas. Lentinan was less effective in the Lewis lung carcinoma and more effective in the M109 model. When the M109 tumor was surgically resected, lentinan improved survival. Lentinan was not effective when B16 melanoma cells were employed. This study again demonstrates the importance of host-tumor relationships and host genetics in the mediation of antitumor activity. The implication of these studies to the employment of glucan in clinical disease states remains to be ascertained.

In studying the potential mechanisms by which lentinan exerts anti-tumor activity, Masuko et al. [24] reported that delayed hypersensitivity reaction, which was markedly decreased in MM46 mammary carcinoma tumor-bearing mice, was enhanced after lentinan administration. Enhancement of cell-mediated immunity is obviously an important factor in anti-tumor effect of glucans.

Abe et al. [1] have recently reported the employment of combination therapy with lentinan, bacterial lipopolysaccharides, and OK432, a preparation derived from *Streptococcus haemolyticus*. It was observed that when multi-modality therapy

was employed, almost complete regression of the MH134 tumor was observed. Those mice which underwent tumor regression showed augmented delayed hypersensitivity reaction, again stressing the importance of enhanced cell-mediated immunity. The mice which underwent combined immunotherapy were also resistant to subsequent challenge with MH134. They did not possess cytolytic antibodies in their serum, indicating the absence of a humoral effect. Studies such as Abe et al. [1] indicate the potential future utilization of multiple immunomodulating agents which may affect different parts of the immune response to maximize therapeutic effectiveness.

Miyaji et al. [26] have studied combination therapy, i.e., immunomodulators with local radiation in the treatment of syngeneic MM46 tumors. The MM46 is a transplantable ascites tumor from a spontaneous mammary adenocarcinoma. When PSK was administered after local radiation, significant inhibition of tumor growth was manifested with an enhanced 60 day survival. No inhibition of tumor growth was observed when PSK was given before irradiation. Thus, as in the case of chemoimmunotherapy, the timing of administration is critical in achieving therapeutic goals. In additional support of the concept of mult-modality therapy, Herlyn et al. [16] have reported that lentinan, in the presence of monoclonal antibodies, would increase the cytotoxic effect of murine peritoneal macrophages against human tumor cells. Maximum cytotoxic activity of macrophages was obtained five days following the intraperitoneal administration of lentinan. Thus, glucan immunomodulation may be more effectively employed in the presence of monoclonal antibodies directed against tumor-specific antigens.

Matsuo et al. [25] studied the antitumor effect of SPG in allogeneic and syngeneic models. In accord with the observations with lentinan [8], SPG showed a significant reduction in anti-tumor activity in neonatally thymectomized mice which were treated with antithymus globulin. T-lymphocytes may play a role in the anti-tumor action of SPG and lentinan, but not of yeast glucan. SPG exhibited significant antitumor activity against a variety of syngeneic murine tumors such as adenocarcinoma 755, MM46, mammary carcinoma, and sarcoma 180 [25]. Thus, essentially uniform data have been obtained denoting that a variety of glucans derived from different sources and of varying structures and molecular weights possess the ability to exert a significant effect on syngeneic murine tumors.

Sugawara et al. [44] have studied various characteristics of SPG-treated macrophages. Although SPG activates macrophages, it was also mitogenic to lymphocytes. Mashiba and Matsunaga [23] have also reported the activation of human monocytes as well as cytostatic and cytotoxic activity by SPG. The most pronounced cytostatic and cytotoxic activity against syngeneic and allogeneic tumor cells was observed when peritoneal macrophages were obtained four days following a single intraperitoneal injection of SPG. Sugawara et al. [44] also observed that larger macrophages exhibited greater antitumor activity than the smaller-sized population of macrophages, which were probably less activated. The importance of macrophages to antitumor activity of SPG was demonstrated by using carrageenan and silica. Treatment of peritoneal cells with carrageenan or silica eliminated their cytotoxic activity against various tumor cell lines. No evidence was found that SPG induced lymphocytes to secrete lymphokines. SPG, like yeast glucan and lentinan induced the production of interleukin-1 from

macrophages. The elevation in IL-1 may be of significance in initiating glucan's immunobiologic activity.

Yanaki et al. [46] have attempted to correlate that antitumor activity of SPG to its molecular structure. Fractions ranging in weight from 5×10^3 to 1.3×10^5 were prepared and their antitumor activity evaluated employing the sarcoma 180 tumor model. Intrinsic viscosities and gel-filtration chromatograms were also obtained as indices of structure and associated with antitumor activity. Antitumor activity was demonstrated with molecular weights of SPG higher than 9×10^4 and reduced or non-existent when molecular weights were less then 10^4. With molecular weights below 10^4, triple helical confirmational structures were not found. However, with molecular weights of 9×10^4 and above, SPG existed in the form of a triple helix, like other macromolecular glucans. Yanaki et al. [46] concluded that the antitumor activity of SPG is related to its triple helical formation. Thus, structure as well as the unique β-1, 3 glucosidic linkage are important facets of the immunopharmacologic activity of glucans.

Janiak et al. [19] studied the elimination of ^{75}selenomethionine-labeled 3LL tumor cells from murine lungs. Employing a variety of immunomodulators, including glucan from *S. cerevisiae*, Janiak et al. [19] observed that an increased rate of elimination of labeled tumor cells occurred 4–6 days following intravenous administration of glucan. Activity was lost 6–8 days later. Intraperitoneal administration of glucan and other biologic response modifiers such as *P. granulosum*, KP-45, or pyran copolymer, produced minimal results denoting that the route of administration is of significance in the mediation of immunobiologic activity.

It was also observed that the increased elimination of labeled tumor cells induced by glucan was modifiable by the administration of cyclophosphamide. Janiak et al. [19] suggested that the effector cells responsible for the clearance of radiolabeled tumors may be related to natural killer (NK) cells. Previous studies of Proctor et al. [35] clearly demonstrated enhanced removal of labeled tumor cells from the lungs of animals which were pretreated with glucan. Proctor et al. [35–37] concluded that the enhanced elimination of tumor cells from lung by glucan treatment was associated with macrophage elements. Indeed, Proctor et al. [36] also demonstrated that the reduced incidence of pulmonary metastases following the intravenous administration of tumor cells correlated well with the enhanced rate of elimination from tumor cells in glucan-treated mice.

Recently Nakajima et al. [27] have reported the preparation of a new antitumor polysaccharide DMG, a degraded D-manno-D-glucan obtained from the culture fluid of *Microellobosporia grisea*. These authors observed the mannan/glucan complex significantly inhibited the growth of a murine syngeneic mammary carcinoma. Mice in which the tumor had completely regressed also showed specific antitumor resistance. Enhanced tumor-specific delayed hypersensitivity reaction was also observed in DMG-treated mice. Spleen cells obtained from DMG-treated mice, when employed in the Winn assay, were found to be tumor-specific and significantly reduced tumor-incidence. The level of antitumor antibody was not influenced by DMG administration. The authors concluded that DMG exerts its antitumor effect through cell-mediated events rather than humoral-mediated activity.

Abe et al. [2] also reported on properties of DMG as compared to the polysaccharides, lentinan, and TAK, a linear glucan obtained from *Alcaligenes faecalis*. The effects of the intratumoral administration of these various polysaccharides was studied at 4, 7, and 10 day intervals. They found that DMG exerted a significant local effect, which was dose dependent, as reflected by inflammatory responses. However, no direct correlation was observed between antitumor activity of the various polysaccharides tested and the degree of inflammation induced.

Ohno et al. [28] investigated the antitumor activity and structural features of glucans isolated from the fruit bodies of *Grifola frondosa*. Water soluble and water insoluble glucans were utilized in this study. Both water soluble and insoluble fractions exerted antitumor activity against sarcoma 180, although the water-soluble fraction had the greated activity. Antitumor activity was correlated with the content of β-1,3 glucans.

Sasaki et al. [41] studied the influence of serum derived from mice injected with TAK-N, a glucan produced from *Alcaligenes faecalis* var. myrogenes, or its carboxymethyl derivative designated (CM-glucan). They observed that serum obtained from mice injected with these glucans would render normal resting macrophages cytotoxic to L5178Y lymphoma cells. The serum factor which induced this cytotoxic activity was found to appear at 2–4 days after the administration of TAK-N and 9–10 days after the administration of its carboxymethyl derivative (CM-glucan). Glucans alone did not induce cytotoxicity of normal peritoneal macrophage in vitro. This finding is in contrast to yeast glucan which will induce cytotoxicity of normal resting peritoneal macrophages. The fractionation of mouse serum indicated two possible factors mediating cytotoxicity, a peptide (4,500) and a peptidoglycan of approximately 9000 daltons. The secretion by macrophages of cytostatic/cytotoxic factors may be an additional mechanism of the expressed anti-tumor activities of glucan.

Immunobiologic Changes Induced by Glucans

Barlin et al. [3] have studied the influence of such agents as *C. parvum*, pyran copolymer, and yeast glucan on prostaglandin E (PGE) release, tumor cytotoxicity, and lymphocyte stimulation. All three agents induced tumor cytotoxic macrophages and enhanced macrophage populations in spleen. It was observed, however, that a differential effect on PGE release existed. In contrast to marked elevations in PGE release induced by *C. parvum*, the influence of yeast glucan and pyran copolymer on PGE release was minimal. These studies again demonstrate differential responses of macrophages to diverse immunomodulators which permit dissection of discrete aspects of host-defense mechanisms and their importance in control of infections and neoplastic diseases.

The mechanisms by which particulate glucan produces profound hyperplasia and hypertrophy in macrophage elements and an associated leukocytosis was evaluated by Way et al. [45]. This group investigated the potential role of archidonic acid metabolites in mediating the cellular responses to glucan. Two approaches were employed in this study; (1) rats were depleted of archidonic acid by employing diets deficient in essential fatty acids, and (2) by pretreatment with the

fatty acid cyclooxygenase inhibitor, indomethacin. Way et al. [45] observed that both treatment interventions markedly reduced hepatic Kupffer cell proliferation, granuloma formation, as well as the leukocytosis induced by glucan. When the essential fatty acid deficient rats were supplemented with ethyl arachidonate, the cellular responses to glucan were restored. Thus, arachidonate acid metabolites may play a role in the proliferative and granulomatous responses following particulate glucan administration.

Mashiba and Matsunaga [23] have reported that SPG possess the capability of activating human peripheral blood monocytes in vitro. Acid phosphatase activity increased in relation to SPG dose. It was also observed that cytostatic activity of macrophages was enhanced when co-cultured with SPG. This event has been universally observed when other glucans were employed.

Kato et al. [20] have reported that PSK would increase serum complement levels in guinea pigs. This study is in agreement with that reported by Glovsky et al. [15] which demonstrated that yeast glucan possessed the ability not only to activate but elevate a variety of complement components. Mannan was relatively inactive denoting that the biologic activity of zymosan is due to its glucan component. Similar observations of complement activation were made utilizing lentinan [8]. The importance of activation as well as elevation of serum complement levels in the complex mechanisms of enhanced host defense mediated by glucan is yet to be fully established [17].

One of the initiating mechanisms of host defense mediated by macrophage activation by glucans is the secretion of interleukin-1 (IL-1). Fruehauf et al. [14] have reported that lentinan will enhance IL-1 production by human monocytes. Lentinan had no direct effect on thymocyte proliferation. Optimal effects in the production of interleukin-1 by monocytes in vitro were seen at doses of approximately 0.1 μg/ml. Chihara has recently reported that interleukin-1 is profoundly elevated one day after the administration of 10 mg/kg of lentinan and gradually declines after that time [8]. No elevation of IL-1 activity was seen when lentinan was administered to athymic nude mice. These findings suggest that lentinan requires enhanced T cell function as a requirement for a number of its activities [8]. This finding appears to be different from that of yeast glucan which exerts its antitumor effect in nude mice, which again stresses the fact that various glucans prepared from divergent sources have different biologic activities.

Conclusion

The extensive and varied reviews regarding the broad spectrum activity of the immunopharmacologic activities of glucans demonstrate the growing interest in these unique molecules. Since the end metabolite is glucose, a new dimension in pharmacology and therapeutics employing this unique molecule is clearly indicated.

At present Krestin is a prescription drug in Japan with both lentinan and Schizophyllan soon to be introduced for the treatment of neoplasia. In the US, clinical studies employing our glucans are presently underway. Mannozym, a glucan-mannan complex, is extensively employed in Hungary and Russia, while

soluble yeast glucan is under clinical investigation in Czechoslovakia. In contrast to all of the previously employed immunomodulators such as BCG, *C. parvum*, MER, pyran copolymer, glucans have few toxic side effects and equivalent or greater immunopharmacologic activities.

Based upon the previously presented data and extensive reviews, as well as current findings from a variety of laboratories, it is clear that glucans are a unique class of immunomodulators and adjuvants with significant clinical potential in infectious diseases, neoplasia, radiation recovery, vaccine development, and control of hemopoietic activity. While significant research is clearly yet to be undertaken regarding the most appropriate clinical employment of glucans, the possible applications in clinical as well as agricultural medicine are both numerous and obvious. Additionally, glucans should be effectively employed in defining the relative contribution of various host cells and their secretory products in host defense mechanisms.

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