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(1→3)-β-D-Glucans as biological response modifiers: a review of structure–functional activity relationships

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(1→3)-β-D-Glucans that have β-D-glucopyranosyl units attached by (1→6) linkages as single unit branches enhance the immune system systemically. This enhancement results in antitumor, antibacterial, antiviral, anticoagulatory and wound healing activities. The (1→3)-β-D-glucan backbone is essential. The most active polymers have degrees of branching (DB) between 0.20 and 0.33. Data suggest both that triple helical structures formed from high molecular weight polymers are possibly important for immunopotentiating activity and that activity is independent of any specific ordered structure. Other data indicate that it is the distribution of the branch units along the backbone chain that is responsible for activity. There are data that indicate both that β-D-glucopyranosyl units are required for immunopotentiating activity and that the specific nature of the substituent is unimportant. There are also data that indicate both that the more water-soluble polymers are more active (up to a certain degree of substitution (DS) or DB) and that some insoluble aggregates are more stimulatory than the soluble polymers. The best conclusion at this time is that the immunopotentiating activity of (1→3)-β-D-glucans depends on a helical conformation and on the presence of hydrophilic groups located on the outside surface of the helix. Immunopotentiation effected by binding of a (1→3)-β-glucan molecule or particle probably includes activation of cytotoxic macrophages, helper T cells, and NK cells, promotion of T cell differentiation, and activation of the alternative complement pathway.

(1→3)-β-D-GLUCAN STRUCTURE

A variety of polysaccharides from a variety of sources have the ability to enhance the immune system, i.e., behave as immunomodulators. Pharmacologically they are classified as biological response modifiers (BRM). The most active appear to be branched (1→3)-β-D-glucans, sometimes referred to as (1→3), (1→6)-β-D-glucans, hereafter referred to simply as (1→3)-β-glucans, to which this brief review is limited.[a] All have a common structure, a main chain consisting of (1→3)-linked β-D-glucopyranosyl units along which are randomly dispersed single β-D-glucopyranosyl units attached by 1→6 linkages, giving a comb-like structure, but the fine structures and conformations of these polymers vary, as do their activities (Wagner *et al.*, 1988; Jamas *et al.*, 1991; Kraus & Franz, 1992). All have significant antitumor activity, a result of activation of the host's immune system, rather than direct cytotoxicity. The most active come from the mycelia, fruiting bodies and culture fluids of fungi (Table 1).

(1→3)-β-D-Glucans are effective against allogeneic, syngeneic, and even autochthonous tumors (Adachi *et al.*, 1987; Chihara *et al.*, 1987; Nanba & Kuroda, 1987*a*; Ohno *et al.*, 1987*b*; Kraus & Franz, 1991) and, in addition, exhibit antibacterial, antiviral, and anti-coagulatory effects. Some have demonstrated wound healing activity (Wagner *et al.*, 1988; Ohno *et al.*, 1990; Jamas *et al.*, 1991). Administration results in hypertrophy of organs and tissues of the reticuloendothelial system and hyperphagocytosis.

Polysaccharide antitumoral activity has been evaluated most often against allogeneic sarcoma 180 in CD-1 mice, a tumor sensitive to immunomodulating

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[a] Only a few selected papers from the voluminous literature on (1 → 3)-β-D-glucans as BRM are presented, with an emphasis on structure–functional activity relationships rather than physiological mechanisms. For earlier reviews on antitumor polysaccharides in general, see Whistler *et al.*, 1976; Witczak & Whistler, 1987.

Table 1. Some (1 → 3)-β-D-glucans with significant antitumor activity and their degrees of branching

Glucan	Source	Approx. DB	Ref.
Pachyman	*Poria cocos*	0.015–0.02	Saito *et al.*, 1968; Hoffman *et al.*, 1971
CI-6P	*Cordyceps cicadae*	0.04	Kiho *et al.*, 1989
	Pythium aphanidermatum	0.06	Blaschek *et al.*, 1992
	Ganoderma applanatum	0.08[a]	Usui *et al.*, 1981
		0.17[a]	Mizuno *et al.*, 1982*a*
Glucan component of zymosan	*Saccharomyces cerevisiae*	0.03–0.2[a]	Manners *et al.*, 1973
GU	*Grifola umbellata*	0.2	Miyazaki *et al.*, 1978
VVG	*Volvariella volvacea*	0.2	Misaki *et al.*, 1986 Kishida *et al.*, 1989
PVG	*Peziza vesiculosa*	0.2	Ohno *et al.*, 1985*b*, 1985*c*
Lentinan	*Lentinus edodes*	0.23, 0.33[a]	Sasaki & Takasaka, 1976
H-3-B	*Cryptoporus volvatus*	0.25	Kitamura *et al.*, 1994
Glomerellan	*Glomerella cingulata*	0.25	Gomaa *et al.*, 1991
HA	*Pleurotus ostreatus*	0.25	Yoshioka *et al.*, 1985
AM-ASN	*Amanita muscaria*	0.3	Kiho *et al.*, 1992
AS-I	*Cochliobolus miyabeanus*	0.3	Nanba & Kuroda, 1987*b*
T-4-N	*Dictyophora indusiata*	0.3	Hara *et al.*, 1983, 1986, 1991
P-I	*Laetisaria arvalis*	0.3	Aouadi *et al.*, 1991
Scleroglucan	*Sclerotium glucanicum*	0.3	Rinaudo & Vincendon, 1982
Grifolan	*Grifola frondosa*	0.31–0.36[a]	Ohno *et al.*, 1985*a*, *d*, 1986*a*; Nanba *et al.*, 1987
CW II, HW II	*Ganoderma lucidum*	0.33, 0.06[a]	Sone *et al.*, 1985
PHYT-G, A1, glucan P	*Phytophthora parasitica*	0.33	Fabre *et al.*, 1984 Blaschek *et al.*, 1987 Bruneteau *et al.*, 1988 Perret *et al.*, 1992
Schizophyllan	*Schizophyllum commune*	0.33	Tabata *et al.*, 1981
Tylopilan	*Tylopilus felleus*	0.33	Defaye *et al.*, 1988
CO-1	*Cordyceps ophioglossoides*	0.375	Yamada *et al.*, 1984 Ohmori *et al.*, 1988
SSG	*Sclerotinia sclerotiorum* IFO 9395	0.5	Ohno *et al.*, 1986*b*
PGG	*Saccharomyces cerevisiae*	0.5	Jamas *et al.*, 1991
	Fomes pinicola	0.6	Maeda *et al.*, 1975 Mizuno *et al.*, 1982*b* Misaki (1984)
Pestalotan	*Pestalotia* sp. 815	0.67	Misaki *et al.*, 1984
β-glucan I	*Auricularia auricula-judae*	0.75	Sone *et al.*, 1978 Misaki *et al.*, 1981

[a] Different plant parts, culture fractions, glucan fractions, or extracts based on different solubilities.

compounds (Nanba & Kuroda, 1987*a*; Ohno *et al.*, 1987*b*). Of the polysaccharides with immunomodulating capacity, only those which consist of a (1→3)-linked β-glucan backbone with (1→6)-linked β-D-glucopyranosyl units as branches produce complete inhibition of tumor growth. (1→3)-β-Glucans from fungi commonly have a tumor inhibition percentage of 99–100%, while other polysaccharides exhibit 10–40% inhibition[b]. Contradictory data exist on the influence of molecular weight, degree of branching (DB), conformation, and intermolecular associations of β-glucans on antitumor activity and on the mechanism(s) of their action as BRM.

Most of the (1→3)-β-glucans with BRM activity have been isolated from Basidiomycetes; a few with pronounced antitumor activity have come from Ascomycetes and Oomycetes. Lentinan, a cell wall glucan from *Lentinus edodes*, and schizophyllan, an extracellular polysaccharide from *Schizophyllum commune*, both of the Basidiomycete family, the two most studied immunomodulating (1→3)-β-D-glucans, and both of which are used as immunotherapeutic agents, consist of a main chain of (1→3)-linked β-D-glucopyranosyl units with β-D-glucopyranosyl branch units linked 1→6 at, on average, an interval of three main chain units (DB 0.33) and have average molecular

[b] The rate of tumor inhibition is expressed as a percentage by weighing the control (untreated) tumor and subtracting the weight of the treated tumor, then dividing this value by the weight of the control tumor and multiplying by 100 to get a percentage ($(C - T)/C \times 100$ = % inhibition), also termed the 'inhibition ratio'.

weights of 500 000 and 450 000, respectively (Kraus & Franz, 1991; Misaki *et al.*, 1993). There is evidence for a regular arrangement of β-D-glucopyranosyl side units at every third main chain unit of the (1→3)-linked backbone of schizophyllan. Fungal (1→3)-β-glucans differ with respect to their branch distribution and DB (Table 1).

The phytopathogenic fungus *Phytophthora parasitica* (class Oomycetes) produces several (1→3)-β-glucans (molecular weights (MW): 10 000, 20 000–80 000, and 200 000) (Bruneteau *et al.*, 1988). These glucans have occasional disaccharides (β-Glc*p*-(1→3)-β-Glc*p*) as branch units. Trisaccharide side chains are present in the highest molecular weight fraction, which also has the highest antitumor activity when tested against the allogeneic sarcoma 180 in CD-1 mice (Perret *et al.*, 1992). The active (1→3)-β-glucan from *Glomerella cingulata* also has mono-, di-, and trisaccharide branch units in decreasing order of abundance (Gomaa *et al.*, 1991).

STRUCTURE–ACTIVITY RELATIONSHIP

Schizophyllan and lentinan have single β-D-glucopyranosyl unit branches at *O*6 of backbone chain units with a DB of 0.33. Other biologically active β-glucans with both higher (0.75) and lower DB (0.04) exist (Table 1); those with DB between 0.20 and 0.33 seem to be the most active. That from *Cryptoporus volvatus* (Basidomycetes) (DB 0.25) had *c.* 94% of the sarcoma 180 growth-inhibiting activity of schizophyllan (Kitamura *et al.*, 1994). The highly branched glucans of *Auricularia auricula-judae* (Kikurage) (DB 0.75) (Sone *et al.*, 1978; Misaki *et al.*, 1981) and of *Pestalotia* sp. 815 (DB 0.67) (Misaki *et al.*, 1984) have limited activity against tumors. The much less branched glucan extracted from *Ganoderma lucidum* with an alkaline solution without heating (DB 0.06) is also only weakly active (Misaki *et al.*, 1993).

Evidence suggests that the activity of these polysaccharides is also dependent on their size, with high molecular weight (100 000–200 000) fractions being most active, while fractions from the same source with molecular weights of 5000–10 000 show no activity (Blaschek *et al.*, 1992; Fabre *et al.*, 1984; Kojima *et al.*, 1986). A (1→3)-linked β-glucanoheptasaccharide is the smallest fragment that competes for the glucan receptor on neutrophils (Janusz *et al.*, 1989) and has been synthesized (Sharp *et al.*, 1984). The fact that there are polysaccharides with different chemical structures, but all of which have immunomodulating activity (Whistler *et al.*, 1976), suggests that the immune response is in part non-specific, determined by size rather than by chemical structure.

(1→3)-β-Glucans have specific molecular structures (Ohno *et al.*, 1988*a*). There are data that suggest that higher ordered structures (triple helices) of high molecular weight (1→3)-β-glucans are responsible for their immunomodulating activity (Hamuro *et al.*, 1971; Norisuye, 1985; Ohno *et al.*, 1987*a*; Kojima *et al.*, 1986; Maeda *et al.*, 1988). There are also data that indicate that it is the distribution of the single glucosyl units along the backbone chain that confers immunomodulating activity (Misaki *et al.*, 1993). Only higher molecular weight (>90 000) molecules appear to form triple helical structures, which are stabilized by the β-D-glucopyranosyl branch units (Saitô *et al.*, 1977, 1991).

Lentinan fibers contain a hexagonal unit cell with dimensions $a = b = 15.8$ Å and c (fiber repeat) = 6 Å (Bluhm & Sarko, 1977). Although five probable models fit this data, all but two triple helical models can be eliminated. The two triple helices differ only in chirality. Both the right-handed and left-handed models are stabilized by interstrand *O*(2)-*O*(2) hydrogen bonds. The triple helical structure of native lentinan consists of three right-handed helices. High molecular weight (>100 000) schizophyllan forms a similar triple helix with a helical symmetry of $P6_3$ and it is reported that only triple helical schizophyllan is active (Kojima *et al.*, 1986). Lower molecular weight (<50 000) schizophyllan has neither the triple helix structure nor any antitumor activity. There are also data that suggest that both lentinan and schizophyllan are active only when they exist in a single helical structure (Saitô *et al.*, 1977, 1991). The single β-D-glucopyranosyl units which protrude from the backbone chain may also stabilize the right-handed single helix.

(1→3)-β-Glucans with an average MW of <20 000 and no ordered structure show great antitumor activity if the DB is <0.25 (Blaschek *et al.*, 1992). The antitumor activity of glomerellan, a (1→3)-β-glucan with a high MW (~670 000) isolated from the culture filtrate of *Glomerella cingulata*, appears to be independent of the presence of ordered structures, since aggregates of triple helices, free triple helices, and a single helical conformation showed comparable activity against sarcoma 180 (Gomaa *et al.*, 1992). *Pythium aphanidermatum* glucan, a mixture of polysaccharides with molecular weights of 10 000 and 20 000 and DB 0.20 and 0.08, respectively, has antitumor activity but no ordered structure (Blaschek *et al.*, 1992; Gomaa *et al.*, 1992; Kraus & Franz, 1992).

A genetically engineered *Saccharomyces cerevisiae* (1→3)-β-glucan known as PGG glucan or Betafectin® has a DB of 0.5. The increased branching on the PGG glucan (over the wild-type yeast glucan, DB 0.2) results in weaker interchain associations and structures with large single helical areas. The structural difference produces a 35-fold higher affinity of the PGG glucan for the β-glucan receptor of human monocytes and neutrophils (Jamas *et al.*, 1991).

(1→3)-β-Glucans from various *Phytophthora* species

with molecular weights of only 20 000 and no helical conformation were active against sarcoma 180, the activity being correlated with the degree of branching (Kraus *et al.*, 1992). The importance of the (1→3)-linked β-glucan backbone to the expression of antitumor activity, and the influence of glycosyl units attached to *O*6 has been investigated (Demleitner *et al.*, 1992*a*). Curdlan, an unbranched (1→3)-β-glucan, and lichenan, a linear mixed-linkage (1 → 3, 1 → 4)-β-D-glucan with 33% (1→3) and 66% (1 → 4) linkages, were modified with β-D-glucopyranosyl, α-L-arabinofuranosyl, α-L-rhamnosyl, and β-gentiobiosyl units added to *O*6. Curdlan modified with D-glucosyl, L-rhamnosyl, L-arabinosyl and, to a lesser extent, gentiobiosyl branch units significantly inhibited sarcoma 180 tumors. However, only D-glucosyl-modified lichenan showed significant antitumor activity; the other lichenan derivatives showed little or no antitumor activity and, in some cases, stimulated tumor growth, indicating the importance of the (1→3)-β-glucan backbone. Modified (1→3)-β-glucans containing D-arabinofuranosyl or D-mannopyranosyl branch units exhibited high antitumor activity, while similarly modified (1 → 4)-β-glucans had no antitumor activity (Matsuzaki *et al.*, 1986). These results suggest that the (1→3)-linked backbone structure is essential, i.e., antitumor activity is absent when the polymer is unbranched (curdlan), but can be induced by branching with glycosyl units. In substituted curdlans, a specific glucosyl substituent was not important for antitumor activity because all derivatives showed a significant antitumor effect.

Sulfonylation (Demleitner *et al.*, 1992*b*), sulfation (Suzuki *et al.*, 1991*a*; Williams *et al.*, 1992), phosphorylation (Di Luzio *et al.*, 1979; Williams *et al.*, 1991), or carboxymethylation (Sasaki *et al.*, 1979; Ohno *et al.*, 1988*b*) of active (1→3)-β-glucans to increase their solubility maintained or increased their antitumor activity. Carboxymethylated SSG maintained potent antitumor activity with degrees of substitution up to 0.14, but activity decreased with progressively higher DS products (Sasaki *et al.*, 1979; Ohno *et al.*, 1988*b*). The reason for this is unclear; electrostatic binding to a receptor and/or ligand, a more active conformation, and increased solubility have been suggested.

Curdlan etherified with sulfonylethyl, sulfonylpropyl, and sulfonylbutyl groups (Demleitner *et al.*, 1992*b*) had significant antitumor activity against allogeneic sarcoma 180 (Kraus & Franz, 1992). The sulfonylalkyl derivatives had MW >300 000; the glycosidically branched derivatives had MW ~25 000. No derivatives of lichenan demonstrated any significant antitumor activity, except for the lichenan modified with β-D-glucopyranosyl units (DB 0.08) (Demleitner *et al.*, 1992*a*, *b*), again indicating the importance of the (1→3)-linked backbone.

Curdlan and lichenan derivatives were evaluated for their mitogenic activity, induction of macrophages, and enhancement of phagocytosis (Kraus & Franz, 1992). In these immunological tests, performed on LPS-low responder C3H/HeJ mice, the antitumor-inactive lichenan derivatives exhibited high immunostimulating effects *in vitro*. Lichenan itself had no effect on immune cells. In a complete reversal of the antitumor assay results, the curdlan derivatives showed no immunostimulating abilities. In fact, phagocytosis of zymosan, a yeast cell wall polysaccharide preparation containing α-D-mannans and β-D-glucans, was inhibited by the curdlan derivative owing to a blocking of the macrophage (1→3)-β-glucan receptor (Czop, 1986).

LC-33, a (1→3)-β-glucan obtained by fractionation of *Lentinus edodes* polysaccharides, was only slightly soluble in water and quite effective against mouse sarcoma 180 (80–98% inhibition) (Chihara *et al.*, 1969). It had no toxic effects, and nothing remained after complete regression of the tumor. When 42 different glycans were tested for their ability to stimulate macrophages, no detectable patterns relating chemical structure to stimulatory activity were observed (Sejelid *et al.*, 1981). However, most had little or no branching. No water-soluble glycans were markedly stimulatory, while some, but not all insoluble glycans showed a potent stimulatory effect. The strongest stimulants were (1→3)-β-glucans and block polymers with (1→3)-linked segments. In a test for the insolubility requirement (indicated not to be necessary by the modification studies) for the stimulatory activity of glycans, laminaran, an unbranched, water-soluble, low molecular weight (1→3)-β-glucan, had no effect. After crosslinking, laminaran became equally stimulatory as yeast glucan, and the addition of soluble laminaran did not inhibit the stimulatory effect of either yeast glucan or the crosslinked laminaran (Seljelid *et al.*, 1981). Crosslinking may have (1) generated a large enough polymer for stimulatory activity; and/or (2) generated the necessary branch-type structure. When lymphocytes were removed, the glycans still stimulated macrophages. When macrophages were separated from glycan-stimulated culture cells, the cells did not remain stimulated, which demonstrated that glycans act directly on macrophages. Patterns of stimulation also differed; yeast glucan and crosslinked laminaran effected a constant increase in macrophage activity, while lichenan caused an initial stimulation, which then subsided after 4 days. Macrophages stimulated by crosslinked laminaran and yeast glucan were clustered, enlarged, and moderately vacuolated, while those stimulated by lichenan were less clustered and more vacuolated.

Controlled periodate oxidation of several (1→3)-β-glucans (only the branch units are oxidized), followed by reduction to produce glucan polyols, produced an enhancement of antitumor activity (Misaki *et al.*, 1981, 1984). The potency of the antitumor polyols was directly related to their polyol content. Complete

removal of the polyol side chains resulted in loss of antitumor activity. The influence of the shape of the D-glucopyranosyl side chains was examined by converting them into the 3,6-anhydro derivative (Kishida *et al.*, 1992; Misaki *et al.*, 1993). The presence of 3,6-anhydro-β-D-glucopyranosyl units not only resulted in loss of the original antitumor activity of the glucan, but in some instances stimulated growth of sarcoma 180 solid tumor cells. In conclusion, removal or modification of the D-glucosyl side chains may result in elimination of antitumor activity. The enhanced activity that results from conversion of the branch units into polyols may be due to the increased water solubility of the glucan. In addition, the presence of numerous polyhydroxylated groups on the periphery of a helical structure may enhance its immunopotentiating activity.

Curdlan, a linear (1→3)-linked β-glucan with no side units, was treated with epichlorohydrin (DS 0.14) (Kishida *et al.*, 1992; Misaki *et al.*, 1993) to produce epoxylated glucans (presumably, not crosslinked) which displayed no antitumor activity. Treating the epoxylated glucans with sodium hydroxide to produce hydrophilic, glycerol-containing glucans resulted in activity enhancement.

Results published to date, although not unequivocal, suggest that the antitumor activity of (1→3)-β-glucans arises from a helical conformation of the glucan backbone, perhaps triple-stranded, but more importantly on the presence of hydrophilic (polyhydroxylated) groups located on the outside surface of the helix. It also suggests that increased water solubility favors antitumor activity, but that there is a limit to the DB (or DS in modified β-glucans) that can be used to produce solubility. The location of substituent groups is probably also important but has not been studied.

BIOLOGICAL ACTIVITY AND CLINICAL USAGE

Clinical use of schizophyllan and lentinan as immunotherapeutic agents for cancer treatment has been practised in Japan since 1986. They are used in conjunction with chemotherapy or radiotherapy. Clinical studies have demonstrated that administration of schizophyllan, along with antineoplastic drugs, prolongs the lives of patients with lung or gastric cancers (Furue, 1987). Schizophyllan is also being used for the immunotherapy of stage II or III cervical cancers in combination with radiotherapy. Use of lentinan in a combined treatment of patients with advanced or recurrent gastric or colorectal cancer has also resulted in an increased lifespan (Chihara *et al.*, 1987). The immunological effects of schizophyllan provide protection against bacterial infections in animals, streptococcal infection in fish, and Sendai virus infections in mice (Furue, 1987).

Since lentinan was first used against transplanted mouse tumors (Maeda & Chihara, 1971), much work has been done to try to understand its mechanism(s) of action, i.e., how it and other (1→3)-β-glucans increase the immunocompetency of the host. It now seems to be clear that β-(1→3)-glucans activate the immune system systemically and that they do this by a mechanism that differs in some features from that effected by other immunopotentiators (Hamuro & Chihara, 1984). Work continues to determine whether there is one or more than one structure–functional activity relationship and the specific mechanism(s) of activation.

Initially, lentinan showed little effectiveness, primarily because the dosages used were above what was later found to be optimal. Lentinan caused complete regression of sarcoma 180 transplanted into ICR mice at a dose of 1 mg/kg for 10 days, while a larger dose of 80 mg/kg for 5 days yielded no antitumor activity in comparison with untreated control mice (Chihara *et al.*, 1969; Aoki, 1984). In a chronic toxicity test, no side effects were observed in the lowest dosage groups; the other groups showed clinical and histological changes (Aoki, 1984). An LD_{50} of more than 100 mg/kg was found for mice, rats, dogs, and monkeys (intravenous injection) (Aoki *et al.*, 1981).

Oncogenes may be present in all cells of the body in a dormant state, and cancer may result from an immune system that does not become activated when the transformed cells first appear (Reizenstein & Mathé, 1984). The mitogenic activity of (1→3)-β-glucans results in several immune responses. These include increased natural killer (NK) cell activity and T cell-mediated cytotoxicity, proliferative responses of peripheral mononuclear cells to mitogen, a stimulated release of cytokines such as interferons (IFN) and interleukins (IL), and induction of phagocytic activity of neutrophils (Aoki, 1984; Hamuro & Chihara, 1984; Reizenstein & Mathé, 1984). Stimulation of interferon production activates the NK cells of the peripheral mononuclear cells (PMNC), but only after a time delay. A possible explanation is that the effector cells for cytotoxicity may penetrate malignant tissues, which results in their disappearance from the blood, and only when a surplus of effector cells has been boosted by host IFN can the increased population of NK cells be noticed (Aoki, 1984). The number of PMNC in cancer patients, in general, declines significantly with the progression of the disease. Administration of lentinan, *in vitro* and *in vivo*, activated PMNC, while administration of various mitogens did not. Termination of the lentinan dosage resulted in lowered proliferative responses to mitogens. Until recently, lentinan had been considered to be able to return a host's immune system to its normal level (immunorestorative). The proliferative responses to dosages of lentinan demonstrate that it is clearly able to augment the immune system above normal; therefore, it is more accurately an immunopotentiator.

The addition of lentinan to a mixed lymphocyte

culture stimulated cytotoxic T cells, but only when there was less than an optimal amount of stimulator cells present (Hamuro & Chihara, 1984), suggesting the presence of a certain stimulus that induces effective cell to cell reaction (Aoki, 1984). Data also suggest that B cells, T cells, and adherent monocytes are all essential for *in vitro* lentinan activation of peripheral blood mononuclear cells (PBMC) (Aoki *et al.*, 1981). Lentinan itself augments synthesis of immunoglobulins by PBMC. It appears to affect two subpopulations, suppressor cells and helper T cells (Hamuro & Chihara, 1984). It has been reported to effect recovery and sometimes to heighten the depressed activity of helper T cells in cancer patients, as well as in tumor-bearing mice (Maeda & Chihara, 1971; Haba *et al.*, 1976). Since the immunosuppressed status of cancer patients has been considered to be caused predominantly by the elevation of suppressor T cell activity, lentinan is a potentially useful agent in the defense against the lowered immune status of cancer patients (Fukimoto *et al.*, 1976*a*, 1976*b*; Bean *et al.*, 1979).

The likely mode of immunopotentiation by (1→3)-β-glucans involves activation of cytotoxic macrophages, helper T cells, and NK cells and the promotion of T cell differentiation (Chihara, 1983; Matsuo *et al.*, 1982; Hamuro & Chihara, 1984). Early evidence suggested that lentinan and other antitumor polysaccharides were T cell adjuvants. However, more recent evidence also implicates the involvement of immune system components that do not require T cells (Di Renzo *et al.*, 1991). These include macrophages (Janusz *et al.*, 1986), neutrophils (Morikawa *et al.*, 1985*a*,*b*) and NK cells (Levy *et al.*, 1979; Lotzová & Gutterman, 1979; Di Renzo *et al.*, 1991). Macrophages are one of many critical components in the immune system, co-operation between which is necessary for tumor rejection. Macrophages have a highly selective cycotoxicity towards cancer cells *in vitro*; and there is evidence that they may also destroy malignant cells *in vivo*. T cell competence appears necessary for selection of macrophage resistance, which suggests that these two cell types interact in the intact host in response to a tumor challenge (Hamuro & Chihara, 1984). Such interactions may occur through lymphokines secreted by T cells.

Macrophage stimulation by (1→3)-β-glucans *in vitro* suggests that tumor inhibition and regression may be the result of destruction by macrophages. In many operable solid tumors, this aspect of the host's immune system may be important. In the case in which cells of tumors proliferate faster than the macrophages can destroy them, a stimulated production of macrophages may produce numbers that could halt tumor growth. In the case of surgery to remove as much of the tumor as possible, recruited macrophages may serve to destroy any cancerous cells or neoplasms that remain. In addition to stimulating the production of macrophages in high numbers, (1→3)-β-glucans may also serve to activate or sensitize the macrophages to cells which are not recognized as 'self' by the host.

Mouse serum treated with (1→3)-β-glucans exhibited greater macrophage-mediated cytotoxicity, as well as a reduction in sarcoma 180 cells (Misaki *et al.*, 1993), and a tumor-inhibiting factor that appeared to be either a protein or glycoprotein was found in the serum (Aoki *et al.*, 1981; Misaki *et al.*, 1993). The presence of the tumor-inhibiting factor was further investigated by partially oxidizing the β-glucan and reductively aminating it to a BSA conjugate, which was then placed in rabbits to raise antibodies. Competitive inhibition studies using gentiobiose and laminaribiose showed that the hapten recognized the non-reducing end terminal sequence and the branched (gentiobiosyl) units. Recognition of the tumor-inhibiting factor was confirmed using the antibodies. When mouse serum containing tumor-inhibiting factor was passed through an immunoadsorbent column containing β-glucan antibodies, the tumor inhibiting factor was retained on the column (Tabata *et al.*, 1981).

Neither *Phytophthora* glucan A1, lentinan, or schizophyllan demonstrated any antitumor activity against sarcoma 180 in an *in vivo* experiment with cyclosporin A as a T cell suppressor, which also suggests that an immunocompetent T cell component is necessary for developing antitumor activity (Kraus & Franz, 1991). All these results indicate that lentinan is a T cell-oriented immunopotentiator and, therefore, requires a functioning T cell component for its biological activities and that the action of (1→3)-β-glucans on the host's immune system might be 3-fold: (1) increased helper T cell production; (2) increased macrophage production; and (3) a non-immunological increase of host defense mechanisms through stimulated acute phase proteins and colony stimulating factor (CSF), which in turn effects proliferation of macrophages, PMNC, and lymphocytes and activation of the complement system.

When (1→3)-β-glucan was administered to $B6DF_1$ mice, significant and prompt suppression of splenic and bone marrow NK cell cytotoxicity against YAC-1 target cells (lymphoma A cell line) occurred *in vitro* (Lotzová & Gutterman, 1979). No *in vitro* antitumor cytotoxicity was observed for up to 15 days after glucan injection, demonstrating a long-lasting depression of NK cells. Neither concentration nor differences in target-to-effector cell ratios affected NK cell activity. The mechanism of action of (1→3)-β-glucans on NK cells cannot depend on thymus-dependent functions since congenitally athymic mice showed the same degree of NK cell depression as did thymus-possessing mice. The effect of (1→3)-β-glucan on NK cells could be either direct or could be mediated by macrophage functions, which have been shown to regulate positively or negatively various immunohematological phenomena

and are known to increase in number and activity in glucan-treated mice, or by the presence of other immunoregulatory cells. NK cell depression could also be caused by rapid differentiation of the pre-T cells into mature T cells. Pre-killer T cells are reported to differentiate into cytotoxic effector T cells (mature T cells) after administration of (1→3)-β-glucans (Hamuro *et al.*, 1978*b*). However, this should not be possible in athymic mice. Therefore, the exact function of NK cells in tumor immunity remains to be established.

In order to treat cancer successfully, total destruction of all transformed cells is necessary (Fidler, 1985). Combinations of radiation and chemotherapy offer limited success in the treatment of cancer. However, many solid tumors have low success rates for chemotherapy since the solid tumor is often not well enough vascularized to transport the drug into the tissue, and high interstitial pressures within the tumor limit drug transport from the blood vessels into surrounding tissue (Jain, 1994). Current methods, at best, only reduce some forms of cancer to below detectable levels for a time period. In addition, anticancer drugs have side effects, e.g. myelosuppression causes sepsis which results in death by microbial infection. Therefore, the development of agents capable of enhancing the proliferation of granulocytes and macrophages has been emphasized.

Lentinan can also be used for treatment of infectious diseases (Seljelid *et al.*, 1987). In patients with pulmonary tuberculosis, it was the phagocytic activity of neutrophils to *Mycobacterium tuberculosis* that was enhanced by the addition of lentinan. Migration of PMNC to the site of inflammation is also increased by immunopotentiators. Lentinan also enhances host resistance to *Listeria monocytogenes* through the production of macrophages.

The (1→3)-β-glucan from *Saccharomyces cerevisiae* that has been genetically engineered to increase its immunomodulating activity (PGG glucan) has an increased avidity for monocyte and neutrophil receptors which results in a rapid amplification of host immune defenses through a cascade of monocyte-derived products (Jamas *et al.*, 1991). It enhances monocyte and neutrophil phagocytosis and microbiocidal capacity and increases the production of cytokines, colony stimulating factors, and inflammatory factors. *In vivo*, PGG glucan has a significant protective effect against acute sepsis from a wide variety of bacterial and fungal pathogenic challenges.

Lentinan has been used against viral infections, since it can induce interferon production, and has shown some promise (Hamuro & Chihara, 1984). Viral malignancy has been prevented in a few cases. T cell leukemias and malignant lymphomas brought about by novel type C retroviruses have been prevented by lentinan immunotherapy. Lentinan and other stimulatory glycans activate the C3 alternative complement pathway via converting C3, since pre-incubation of sera with glycans produces complement cleavage products (Hamuro *et al.*, 1978*a*; Wagner *et al.*, 1988). It is possible that intracellular processes following endocytosis of (1→3)-β-glucans effect a complement reaction, producing products that stimulate macrophages.

Phytophthora glucan A1, schizophyllan, and lentinan demonstrated a mitogenic effect on mouse spleen lymphocytes of lipopolysaccharide (LPS)-low responder C3H/HeJ-mice, used to minimize the effect of any endotoxin content, with a significant increase in the number of lymphocytes. When human neutrophil granulocytes (PMN) were incubated with A1, phagocytosis of zymosan was increased 2.5-fold (Allen, 1977; Blair *et al.*, 1988). When tested with mouse peritoneal exudate cells targeted against P-815 tumor cells, A1 induced cytotoxicity in macrophages *in vitro* without γ-interferon as a T cell signal. Lentinan was unable to show any induction of cytotoxic macrophages in this assay (Kraus & Franz, 1991). Antitumor-active (1→3)-β-glucans showed little or no significant increase in lymphocyte proliferation (Kraus & Franz, 1992). Since cytotoxic macrophages play an important role in a host's defense against cancer, the ability of polysaccharides to render macrophages cytotoxic to tumor cells is important (Levy *et al.*, 1979). To test the (1→3)-β-glucans, macrophages were co-incubated with γ-interferon, which serves as a T cell-derived signal. The antitumor-active polysaccharides did not render macrophages cytotoxic against P-815 tumor cells; furthermore, the polymers demonstrated no direct cytotoxic effects against P-815 mastocytoma cells (Kraus & Franz, 1992). In addition, the antitumor-active (1→3)-β-glucans did not produce phagocytic activity (Czop, 1986). Thus, there was no direct effect, which did not require cytokines, of these (1→3)-β-glucans on macrophages.

A major obstacle to the clinical utilization of (1→3)-β-glucans as BRM is their relative lack of solubility in aqueous media. Topical or intralesional administration of an insoluble, microparticulate (1→3)-β-glucan isolated from *S. cerevisiae* induced no toxicity. Intravenous (systemic) administration of the microparticulate form, however, was associated with toxicity which manifested itself as hepatosplenomegaly, granuloma formation, micro-embolization, and enhanced endotoxin sensitivity (Williams *et al.*, 1991). Water-soluble derivatives maintained antitumor activity. Glucan sulfate administered to mice resulted in a biphasic stimulation (50% increase) of the murine femoral bone marrow cells (Williams *et al.*, 1992).

Zymosan, composed mainly of an insoluble yeast β-glucan, activates neutrophils (PMN) through a trypsin-sensitive recognition mechanism (Morikawa *et al.*, 1985*b*; Steadman *et al.*, 1990; Di Renzo *et al.*, 1991). PMN CR3 receptor is believed to be involved (Araki

et al., 1990; Steadman *et al.*, 1990; Di Renzo *et al.*, 1991). Zymosan generated a dose and time-dependent release of the secondary lysosomal granule marker vitamin B_{12} binding protein, leukotriene B_4 (LTB_4), and superoxide from PMN (Morikawa *et al.*, 1985*a*) and was phagocytosed with similar dose-dependent kinetics (Steadman *et al.*, 1990). Both zymosan and its β-glucan component generated a transmembrane signal; however, pre-incubation of PMN with tumour necrosis factor alpha (TNF-α) augmented phagocytosis and LTB_4 and superoxide generation by PMN in response to activation by zymosan. None of the responses to (1→3)-β-glucan was significantly increased after incubation with human recombinant (rh)TNF-α, which suggests that, although there is homology between part of the activating mechanism responsible for the generation of a transmembrane signal in response to both particles, a component of the recognition mechanism for zymosan is distinct from that of (1→3)-β-glucan and this component is then up-regulated in response to rhTNF-α (Steadman *et al.*, 1990).

The human alternative complement pathway proteins are activated by (1→3)-β-glucans that are aggregated; the activated proteins, i.e., protein + (1 → 3)-β-glucan, then exhibited increased uptake by macrophages. Human monocytes recognized (1→3)-β-glucans through a receptor that can be functionally inhibited by structurally similar glucans (Janusz *et al.*, 1986). These glucans resulted in receptor-mediated phagocytosis of zymosan, irrespective of their states of solubility. In the absence of opsonins, human peripheral blood monocytes phagocytosed particulate (1→3)-β-glucan, which activated the alternative complement pathway (Czop & Austen, 1985*a*). β-Glucan receptor-mediated phagocytosis was inhibited by soluble (1→3)-β-glucan, as was leukotriene generation (Czop & Austen, 1985*a*, 1985*b*). Pretreatment of monocytes with barley β-glucan suspensions decreased their phagocytic responses to zymosan particles, rabbit erythrocytes, and yeast glucan particles, but did not affect their Fc, C3b(CR1), or fibronectin receptor-mediated functions (Czop, 1986; Konopski *et al.*, 1991). The (1→3)-β-glucan receptor was not inhibited by α-glucans, galactans or mannans. Binding of (1→3)-β-glucan particles to the receptor stimulated monocytes to metabolize endogenous arachidonic acid to substantial quantities of leukotriene B_4 (Czop & Austen, 1985*b*; Czop, 1986; Steadman *et al.*, 1990). (1→3)-β-Glucan receptors are as prevalent as Fc receptors for IgG on phagocytic cells. It can be concluded that the receptor is an opsonin-independent phagocytic receptor with specificity for particulate activators of the alternative complement pathway.

The (1→3)-β-glucan from *S. cerevisiae* is a potent reticuloendothelial stimulant that augments hemopoiesis *in vivo* and granulopoiesis and macrophage genesis *in vitro* (Lotzová & Gutterman, 1979). In clinical trials, it was effective in reducing the size of malignant lesions, especially those of melanomas, lung carcinomas, and breast carcinomas when administered intralesionally (Mansell *et al.*, 1975). Reduction of the malignant lesions was accompanied by cell necrosis and infiltration of monocytes/macrophages. Non-malignant cell growth in humans was inhibited, but the (1→3)-β-glucan was therapeutically ineffective against various syngeneic tumors in mice and guinea pigs (Mansell *et al.*, 1975).

Oral administration may be important for eliminating the side effects of (1→3)-β-glucans, including the pain that accompanies parenteral administration. *Sclerotinia sclerotiorum* glucan (SSG) exhibited antitumor and immunomodulating activities when administered either orally or parenterally (Suzuki *et al.*, 1991*b*). Its effectiveness as a BRM by oral administration distinguishes it from other glucans (Suzuki *et al.*, 1989, 1990). The mechanism of inhibition by oral administration of SSG is unknown. Possibly, the mucosal immune response system is activated.

Intraperitoneal administration of SSG increased the number of peripheral leukocytes, especially PMN and monocyte-macrophages (Hashimoto *et al.*, 1990), enhanced non-specific effector activities (Suzuki *et al.*, 1988), and exhibited antitumor activities against syngeneic tumor cells, especially a solid form of IMC carcinoma (Suzuki *et al.*, 1988).

REFERENCES

Adachi, K., Nanba, H. & Kuroda, H. (1987). Potentiation of host-mediated antitumor activity in mice by β-glucan obtained from *Grifola frondosa* (maitake). *Chem. Pharm. Bull.*, **35**, 262–270.

Allen, R.C. (1977). Evaluation of serum opsonic capacity by quantitating the initial chemiluminescent response from phagocytizing polymorphonuclear leukocytes. *Infect. Immun.*, **15**, 827–833.

Aoki, T. (1984). Lentinan. In *Immune Modulation Agents and Their Mechanisms*, eds R.L. Fenichel & M.A. Chirigos. Marcel Dekker, New York, pp. 63–77.

Aoki, T., Miyakoshi, H., Horikawa, Y. & Usuda, Y. (1981). Staphage lysate and lentinan as immunomodulators and/or immunopotentiators in clinical and experimental systems. *Prog. Cancer Res. Ther.*, **16**, 101–112.

Aouadi, S., Heyraud, A., Seigle-Murandi, F., Steiman, R., Kraus, J. & Franz, G. (1991). Structure and properties of an extracellular polysaccharide from *Laetisaria arvalis*. Evaluation of its antitumour activity. *Carbohydr. Polym.*, **16**, 155–165.

Araki, A., Inoue, T., Kimura, S., Fukase, S. & Sendo, F. (1990). Enhancement of polymorphonuclear leukocyte-mediated tumor cytotoxicity by serum factor(s). *Jpn. J. Cancer Res.*, **81**, 69–78.

Bean, M.A., Akiyama, M., Kodera, Y., Dupont, B. & Hansen, J.A. (1979). Human blood T lymphocytes that suppress the mixed leukocyte culture reactivity of lymphocytes from HLA-B14 bearing individuals. *J. Immunol.*, **123**, 1610–1614.

Blair, A.L., Cree, I.A., Beck, J.S. & Hastings, M.J.G. (1988). Measurement of phagocyte chemiluminescence in a microtitre plate format. *J. Immunol. Meth.*, **112**, 163–168.

Blaschek, W., Käsbauer, J., Kraus, J. & Franz, G. (1992). *Pythium aphanidermatum:* culture, cell wall composition, and isolation and structure of antitumour storage and solubilised cell-wall (1→3), (1→6)-β-D-glucans. *Carbohydr. Res.*, **231,** 293–307.

Blaschek, W., Schütz, M., Kraus, J. & Franz, G. (1987). *In vitro* production of specific polysaccharides: isolation and structure of an antitumor active β-glucan from *Phytophthora parasitica. Food Hydrocoll.*, **1,** 371–380.

Bluhm, T.L. & Sarko, A. (1977). The triple helical structure of lentinan, a linear β-(1→3)-D-glucan. *Can. J. Chem.*, **55,** 293–299.

Bruneteau, M., Fabre, I., Perret, J., Michel, G., Ricci, P., Joseleau, J.-P., Kraus, J., Schneider, M., Blaschek, W. & Franz, G. (1988). Antitumor active β-D-glucans from *Phytophthora parasitica. Carbohydr. Res.*, **175,** 137–143.

Chihara, G. (1983). Preclinical evaluation of lentinan in animal models. *Adv. Exp. Med. Biol.*, **166,** 189–197.

Chihara, G., Hamuro, J., Maeda, Y.Y., Shiio, T. & Suga, T. (1987). Antitumor and metastasis-inhibitory activities of lentinan as an immunomodulator: an overview. *Cancer Detect. Prev.*, **Suppl. 1,** 423–443.

Chihara, G., Maeda, Y., Hamuro, J., Sasaki, T. & Fukuoka, F. (1969). Inhibition of mouse sarcoma 180 by polysaccharides from *Lentinus edodes* (Berk.) Sing. *Nature,* **222,** 637–638.

Czop, J.K. (1986). The role of β-glucan receptors on blood and tissue leukocytes in phagocytosis and metabolic activation. *Pathol. Immunopathol. Res.*, **5,** 286–296.

Czop, J.K. & Austen, K.F. (1985*a*). Properties of glycans that activate the human alternative complement pathway and interact with human monocyte β-glucan receptor. *J. Immunol.*, **135,** 3388–3393.

Czop, J.K. & Austen, K.F. (1985*b*). Generation of leukotrienes by human monocytes upon stimulation of their β-glucan phagocytosis. *Proc. Natl. Acad. Sci. U.S.A.*, **82,** 2751–2755.

Defaye, J., Kohlmünzer, S., Sodzawiczny, K. & Wong, E. (1988). Structure of an antitumor, water-soluble glucan from the carpophores of *Tylpilus felleus. Carbohydr. Res.*, **173,** 316–323.

Demleitner, S., Kraus, J. & Franz, G. (1992*a*). Synthesis and antitumour activity of derivatives of curdlan and lichenan branched at C-6. *Carbohydr. Res.*, **226,** 239–246.

Demleitner, S., Kraus, J. & Franz, G. (1992*b*). Synthesis and antitumour activity of sulfoalkyl derivatives of curdlan and lichenan. *Carbohydr. Res.*, **226,** 247–252.

Di Luzio, N.R., Williams, D.L., McNamee, R.B., Edwards, B.F. & Kitahama, A. (1979). Comparative tumor-inhibitory and antibacterial activity of soluble and particulate glucan. *Int. J. Cancer,* **24,** 773–779.

Di Renzo, L., Yefenof, E. & Klein, E. (1991). The function of human NK cells is enhanced by β-glucan, a ligand of CR3 (CD11b/CD18). *Eur. J. Immunol.*, **21,** 1755–1758.

Fabre, I., Bruneteau, M., Ricci, P. & Michel, G. (1984). Isolation and structural study on *Phytophthora parasitica* glucans. *Eur. J. Biochem.*, **142,** 99–103.

Fidler, I.J. (1985). Macrophages and metastasis – a biological approach to cancer therapy. *Cancer Res.*, **45,** 4714–4726.

Fukimoto, S., Greene, M.I. & Sehon, A.H. (1976*a*). Regulation of the immune response to tumor antigens. I. Immunosuppressor cells in tumor-bearing hosts. *J. Immunol.*, **116,** 791–799.

Fukimoto, S., Greene, M.I. & Sehon, A.H. (1976*b*). Regulation of the immune response to tumor antigens. II. The nature of immunosuppressor cells in tumor-bearing hosts. *J. Immunol.*, **116,** 800–806.

Furue, H. (1987). Biological characteristics and clinical effect of sizofilan (SPG). *Med. Actual.*, **23,** 335–346.

Gomaa, K., Kraus, J., Franz, G. & Röper, H. (1991). Structural investigations of glucans from cultures of *Glomerella cingulata* Spaulding & von Schrenck. *Carbohydr. Res.*, **217,** 153–161.

Gomaa, K., Kraus, J., Rosskopf, F., Röper, H. & Franz, G. (1992). Antitumour and immunological activity of a β1 → 3/1 → 6 glucan from *Glomerella cingulata. J. Cancer Res. Clin. Oncol.*, **118,** 136–140.

Haba, S., Hamaoka, T., Takatsu, K. & Kitagawa, M. (1976). Selective suppression of T-cell activity in tumor-bearing mice and its improvement by lentinan, a potent anti-tumor polysaccharide. *Int. J. Cancer,* **18,** 93–104.

Hamuro, J. & Chihara, G. (1984). Lentinan, a T-cell-oriented immunopotentiator. In *Immune Modulation Agents and Their Mechanisms*, eds R.L. Fenichel & M.A. Chirigos. Marcel Dekker, New York, pp. 409–436.

Hamuro, J., Hadding, U. & Bitter-Suermann, D. (1978*a*). Solid phase activation of alternative pathway of complement by β-1,3-glucans and its possible role for tumour regressing activity. *Immunology,* **34,** 695–705.

Hamuro, J., Maeda, Y.Y., Arai, Y., Fukuoka, F. & Chihara, G. (1971). Significance of the higher structure of the polysaccharides lentinan and pachymaran with regard to their antitumor activity. *Chem.-Biol. Interact.*, **3,** 69–71.

Hamuro, J., Wagner, H. & Röllinghoff, M. (1978*b*). β-(1→3)-Glucans as a probe for T cell specific immune adjuvants. II. Enhanced *in vitro* generation of cytotoxic T lymphocytes. *Cell. Immunol.*, **38,** 328–335.

Hara, C., Kiho, T. & Ukai, S. (1983). A branched (1→3)-β-D-glucan from a sodium carbonate extract of *Dictyophora indusiata* Fisch. *Carbohydr. Res.*, **117,** 201–213.

Hara, C., Kiho, T. & Ukai, S. (1986). A branched (1→3)-β-D-glucan from a water extract of *Dictyophora indusiata* Fisch. *Carbohydr. Res.*, **145,** 237–246.

Hara, C., Kumazawa, Y., Inagaki, K., Kareko, M., Kiho, T. & Ukai, S. (1991). Mitogenic and colony-stimulating factor-inducing activities of polysaccharide fractions from the fruit bodies of *Dictyophora indusiata* Fisch. *Chem. Pharm. Bull.*, **39,** 1615–1616.

Hashimoto, K., Suzuki, I., Ohsawa, M., Oikawa, S. & Yadomae, T. (1990). Enhancement of hematopoietic response of mice by intraperitoneal administration of β-glucan, SSG, obtained from *Sclerotinia sclerotiorum. J. Pharmacobio-Dyn,* **13,** 512–517.

Hoffmann, G.C., Simson, B.W. & Timell, T.E. (1971). Structure and molecular size of pachyman. *Carbohydr. Res.*, **20,** 185–188.

Jain, R.K. (1994). Barriers to drug delivery in solid tumors. *Sci. Am.*, **271**(7), 58–65.

Jamas, S., Easson, D.D., Jr., Ostroff, G.R. & Onderdonk, A.B. (1991). PGG – Glucans. A novel class of macrophage-activating immunomodulators. *ACS Symp. Ser.*, **469,** 44–51.

Janusz, M.J., Austen, K.F. & Czop, J.K. (1986). Isolation of soluble yeast β-glucans that inhibit human monocyte phagocytosis mediated by β-glucan receptors. *J. Immunol.*, **137,** 3270–3276.

Janusz, M.J., Austen, K.F. & Czop, J.K. (1989). Isolation of a yeast heptaglucoside that inhibits monocyte phagocytosis of zymosan particles. *J. Immunol.*, **142,** 959–965.

Kiho, T., Ito, M., Yosida, I., Nagai, K., Hara, C. & Ukai, S. (1989). Polysaccharides in fungi. XXIV. A (1→3)-β-D-glucan from the alkaline extract of the insect-body portion of Chán hua (fungus: *Cordyceps cicadae*). *Chem. Pharm. Bull.*, **37,** 2770–2772.

Kiho, T., Katsuragawa, M., Nagai, K., Ukai, S. & Haga, M.

(1992). Structure and antitumor activity of a branched (1→3)-β-D-glucan from the alkaline extract of *Amanita muscaria*. *Carbohydr. Res.*, **224,** 237–243.

Kishida, E., Sone, Y. & Misaki, A. (1989). Purification of an antitumor-active, branched (1→3)-β-D-glucan from *Volvariella volvacea*, and elucidation of its fine structure. *Carbohydr. Res.*, **193,** 227–239.

Kishida, E., Sone, Y. & Misaki, A. (1992). Effects of branch distribution and chemical modifications of antitumor (1→3)-β-D-glucans. *Carbohydr. Polym.*, **17,** 89–95.

Kitamura, S., Hori, T., Kurita, K., Takeo, K., Hara, C., Itoh, W., Tabata, K., Elgsaeter, A. & Stokke, B.T. (1994). An antitumor, branched (1→3)-β-D-glucan from a water extract of fruiting bodies of *Cryptoporus volvatus*. *Carbohydr. Res.*, **263,** 111–121.

Kojima, T., Tabata, K., Itoh, W. & Yanaki, T. (1986). Molecular weight dependence of the antitumor activity of schizophyllan. *Agric. Biol. Chem.*, **50,** 231–232.

Konopski, Z., Rasmussen, L.T., Seljelid, R. & Eskeland, T. (1991). Phagocytosis of β-1,3-D-glucan-derivatized microbeads by mouse peritoneal macrophages involves three different receptors. *Scand. J. Immunol.*, **33,** 297–306.

Kraus, J., Blaschek, W., Schütz, M. & Franz, G. (1992). Antitumor activity of cell wall β-1,3/1,6-glucans from *Phytophthora* spp., *Planta Med.*, **58,** 39–42.

Kraus, J. & Franz, G. (1991). β(1 → 3)Glucans: anti-tumor activity and immunostimulation. In *Fungal Cell Wall and Immune Response*, eds J.-P. Latgé & D. Boucias. NATO ASI Series H53, Springer, Berlin, pp. 431–444.

Kraus, J. & Franz, G. (1992). Immunomodulating effects of polysaccharides from medicinal plants. In *Microbial Infections*, eds H. Friedman, T.W. Klein & H. Yamaguchi. Plenum Press, New York, pp. 299–308; *Adv. Exp. Med. Biol.*, **319,** 299–308.

Levy, P.C., Shaw, G.M. & LoBuglio, A.F. (1979). Human monocyte, lymphocyte, and granulocyte antibody-dependent cell-mediated cytotoxicity toward tumor cells. I. General characteristics of cytolysis. *J. Immunol.*, **123,** 594–599.

Lotzová, E. & Gutterman, J.U. (1979). Effect of glucan on natural killer (NK) cells: effector cell activities. *J. Immunol.*, **123,** 607–611.

Maeda, Y.Y. & Chihara, G. (1971). Lentinan, a new immuno-accelerator of cell-mediated responses. *Nature*, **229,** 634.

Maeda, Y.Y., Ishimura, K., Takasuka, N., Sasaki, T. & Chihara, G. (1975). *Host. Def. Cancer Potentiation, Proc. Int. Symp. Princess Takamatsu Cancer Res. Fund, 5th*, 181–197. *Chem. Abstr.*, **84,** 19411r (1975).

Maeda, Y.Y., Watanabe, S.T., Chihara, C. & Rokutanda, M. (1988). Denaturation and renaturation of a β-1,6:1,3-glucan, lentinan, associated with expression of T-cell-mediated responses. *Cancer Res.*, **48,** 671–675.

Manners, D.J., Masson, A.J. & Patterson, J.C. (1973). The structure of a β-(1→3)-D-glucan from yeast cell walls. *Biochem. J.*, **135,** 19–30.

Mansell, P.W.A., Ichinose, H., Reed, R.J., Krementz, E.T., McNamee, R. & Di Luzio, N.R. (1975). Macrophage-mediated destruction of human malignant cells. *J. Natl. Cancer Inst.*, **54,** 571–580.

Matsuo, T., Arika, T., Mitani, M. & Komatsu, N. (1982). Pharmacological and toxicological studies of a new antitumor polysaccharide, schizophyllan. *Arzneim.-Forsch./Drug Res.*, **32,** 647–657.

Matsuzaki, K., Sato, T., Enomoto, K., Yamamoto, I., Oshima, R., Hatanak, K.-I., Uryu, T., Kaku, H., Sone, Y. & Misaki, A. (1986). Synthesis of water-soluble, branched polysaccharides having D-mannopyranose, D-arabinofuranose, or oligo-D-arabinofuranose side-chains and their antitumor activity. *Carbohydr. Res.*, **157,** 171–182.

Misaki, A. (1984). Antitumor polysaccharides. Interrelation between their structures and antitumor effects. *Kagaku to Kogyo* (Osaka), **58,** 407–421. (1985). *Chem. Abstr.*, **102,** 55469q.

Misaki, A., Kakuta, M., Sasaki, T., Tanaka, M. & Miyaji, H. (1981). Studies on interrelation of structure and antitumor effects of polysaccharides: antitumor action of periodate-modified, branched (1→3)-β-D-glucan of *Auricularia auricula-judae*, and other polysaccharides containing (1→3)-glycosidic linkages. *Carbohydr. Res.*, **92,** 115–129.

Misaki, A., Kawaguchi, K., Miyaji, H., Nagae, H., Hokkoku, S., Kakuta, M. & Sasaki, T. (1984). Structure of pestalotan, a highly branched (1→3)-β-D-glucan elaborated by *Pestalotia* sp. 815, and the enhancement of its antitumor activity by polyol modification of the side chains. *Carbohydr. Res.*, **129,** 209–227.

Misaki, A., Kishida, E., Kakuta, M. & Tabata, K. (1993). Antitumor fungal (1→3)-β-D-glucans: structural diversity and effects of chemical modification. In *Carbohydrates and Carbohydrate Polymers*, ed. M. Yalpani. ATL Press, Mount Prospect, Illinois U.S.A., pp. 116–129.

Misaki, A., Nasu, M., Sone, Y., Kishida, E. & Kinoshita, C. (1986). Comparison of structure and antitumor activity of polysaccharides isolated from Fukurotake, the fruiting body of *Volvariella volvacea*. *Agric. Biol. Chem.*, **50,** 2171–2183.

Miyazaki, T., Oikawa, N., Yamada, H. & Yadomae, T. (1978). Structural examination of antitumour, water-soluble glucans from *Grifora umbellata* by use of four types of glucanase. *Carbohydr. Res.*, **65,** 235–243.

Mizuno, T., Iwasaki, Y., Satoh, H., Usui, T., Shinkai, K., Arakawa, M. & Tanaka, M. (1982*a*). Studies on the host-mediated antitumor polysaccharides. V. Chemical structure and antitumor activity of a water-soluble glucan isolated from tsugasarunokoshikake, the fruit body of *Fomes pinicola*. *Shizuoka Daigaku Nogakubu Kenkyu Hokoku*, **32,** 29–40. (1983).*Chem. Abstr.*, **98,** 212584s.

Mizuno, T., Ushiyama, M., Usui, T., Arakawa, M., Shinkai, K. & Shimizu, M. (1982*b*). Studies on the host-mediated antitumor polysaccharides. VI. Isolation and characterization of antitumor active β-D-glucan from mycelial cells of *Ganoderma applanatum*. *Shizuoka Daigaku Nogakubu Kenkyu Hokoku*, **32,** 41–58 (1983). *Chem. Abstr.*, **98,** 212585t.

Morikawa, K., Kamegaya, S., Yamazaki, M. & Mizuno, T. (1985*a*). Hydrogen peroxide as a tumoricidal mediator of murine polymorphonuclear leukocytes induced by a linear β-1,3-D-glucan and some other immunomodulators. *Cancer Res.*, **45,** 3482–3486.

Morikawa, K., Takeda, R., Yamazaki, M. & Mizuno, D. (1985*b*). Induction of tumoricidal activity of polymorphonuclear leukocytes by a linear β-1,3-D-glucan and other immunomodulators in murine cells. *Cancer Res.*, **45,** 1496–1501.

Nanba, H., Hamaguchi, A. & Kuroda, H. (1987). The chemical structure of an antitumor polysaccharide in fruit bodies of *Grifola frondosa* (Maitake). *Chem. Pharm. Bull.*, **35,** 1162–1168.

Nanba, H. & Kuroda, H. (1987*a*). Potentiating effect of β-glucan from *Cochliobolus miyabeanus* on host-mediated antitumor activity in mice. *Chem. Pharm. Bull.*, **35,** 1289–1293.

Nanba, H. & Kuroda, H. (1987*b*). The chemical structure of an antitumor polysaccharide in mycelia of *Cochliobolus miyabeanus*. *Chem. Pharm. Bull.*, **35,** 1285–1288.

Norisuye, T. (1985). Triple-stranded helical structure of schizophyllan and its antitumor activity of aqueous solution. *Makromol. Chem.* (Suppl.), **14,** 105–108.

Ohmori, T., Tamura, K., Wakaiki, A., Kawanishi, G., Tsuru, S., Yadomae, T. & Nomoto, K. (1988). Dissociation of a glucan fraction (CO-1) from protein-bound polysaccharide of *Cordyceps ophioglossoides* and analysis of its antitumor effect. *Chem. Pharm. Bull.*, **36,** 4512–4518.

Ohno, N., Adachi, Y., Ohsawa, M., Sato, K., Oikawa, S. & Yadomae, T. (1987*a*). Conformational changes of the two different conformers of grifolan in sodium hydroxide, urea or dimethyl sulfoxide solution. *Chem. Pharm. Bull.*, **35,** 2108–2113.

Ohno, N., Adachi, Y., Suzuki, I., Sato, K., Oikawa, S. & Yadomae, T. (1985*a*). Structural characterization and antitumor activity of the extracts from matted mycelium of cultured *Grifola frondosa*. *Chem. Pharm. Bull.*, **33,** 3395–3401.

Ohno, N., Adachi, Y., Suzuki, I., Sato, K., Oikawa, S. & Yadomae, T. (1986*a*). Characterization of the antitumor glucan obtained from liquid-cultured *Grifola frondosa*. *Chem. Pharm. Bull.*, **34,** 1709–1715.

Ohno, N., Adachi, Y. & Yadomae, T. (1988*a*). Conformations of fungal β-D-glucans in the fruit body of edible fungi assessed by cross polarization-magic angle spinning carbon-13 nuclear magnetic resonance spectroscopy. *Chem. Pharm. Bull.*, **36,** 1198–1204.

Ohno, N., Kurachi, K. & Yadomae, T. (1988*b*). Physicochemical properties and antitumor activities of carboxymethylated derivatives of glucan from *Sclerotinia sclerotiorum*. *Chem. Pharm. Bull.*, **36,** 1016–1025.

Ohno, N., Mimura, H., Suzuki, I. & Yadomae, T. (1985*b*). Antitumor activity and structural characterization of polysaccharide fractions extracted with cold alkali from a fungus, *Peziza vesiculosa*. *Chem. Pharm. Bull.*, **33,** 2564–2568.

Ohno, N., Mimura, H., Suzuki, I. & Yadomae, T. (1985*c*). Purification, antitumor activity and structural characterization of β-1,3-glucan from *Peziza vesiculosa*. *Chem. Pharm. Bull.*, **33,** 5096–5099.

Ohno, N., Suzuki, I., Sato, K., Oikawa, S., Miyazaki, T. & Yadomae, T. (1985*d*). Purification and structural characterization of an antitumor β-1,3-glucan isolated from hot water extract of the fruit body of *Grifola frondosa*. *Chem. Pharm. Bull.*, **33,** 4522–4527.

Ohno, N., Suzuki, Y., Sato, K., Oikawa, S. & Yadomae, T. (1987*b*). Effect of grifolan on the ascites form of sarcoma 180. *Chem. Pharm. Bull.*, **35,** 2576–2580.

Ohno, N., Suzuki, I. & Yadomae, T. (1986*b*). Structure and antitumor activity of a β-1,3-glucan isolated from the culture filtrate of *Sclerotinia sclerotiorum* IFO 9395. *Chem. Pharm. Bull.*, **34,** 1362–1365.

Ohno, N., Suzuki, T., Saito, K. & Yadomae, T. (1990). Enhancement of clot formation in human plasma by β-glucans. *J. Pharmacobio-Dyn.*, **13,** 525–532.

Perret, J., Bruneteau, M., Michel, G., Marais, M.F., Joseleau, J.P. & Ricci, P. (1992). Effect of growth conditions on the structure of β-D-glucans from *Phytophthora parasitica* Dastur, a phytophathogenic fungus. *Carbohydr. Polym.*, **17,** 231–236.

Reizenstein, P. & Mathé, G. (1984). Immunomodulating agents. *Immunol. Ser.*, **35,** 347–361.

Rinaudo, M. & Vincendon, M. (1982). ^{13}C Nmr structural investigation of scleroglucan. *Carbohydr. Polym.*, **2,** 135–144.

Saito, H., Misaki, A. & Harada, T. (1968). A comparison of the structure of cardlan and packyman. *Agricult. Biol. Chem.*, **32,** 1261–1269.

Saitô, H., Ohki, T., Takasuka, N. & Sasaki, T. (1977). A ^{13}C N.m.r. spectral study of a gel-forming (1→3)-β-D-glucan, (lentinan) from *Lentinus edodes*, and its acid-degraded fractions. Structure and dependence of conformation on the molecular weight. *Carbohydr. Res.*, **58,** 293–305.

Saitô, H., Yoshioka, Y., Uehara, N., Aketagawa, J., Tanaka, S. & Shibata, Y. (1991). Relationship between conformation and biological response for (1→3)-β-D-glucans in the activation of coagulation Factor G from limulus amebocyte lysate and host-mediated antitumor activity. Demonstration of single-helix conformation as a stimulant. *Carbohydr. Res.*, **217,** 181–190.

Sasaki, T., Abiko, N., Nitta, K., Takasuka, N. & Sugino, Y. (1979). Antitumor activity of carboxymethylglucans obtained by carboxymethylation of (1→3)-β-D-glucan from *Alcaligenes-faecalis* var. *myxogenes* IFO 13140. *Eur. J. Cancer*, **15,** 211–215.

Sasaki, T. & Takasaka, N. (1976). Further study of the structure of lentinan, an anti-tumor polysaccharide from *Lentinus edodes*. *Carbohydr. Res.*, **47,** 99–104.

Seljelid, R., Bögwald, J. & Lundwall, A. (1981). Glycan stimulation of macrophages *in vitro*. *Exp. Cell Res.*, **131,** 121–129.

Seljelid, R., Rasmussen, L.T., Larm, O. & Hoffman, J. (1987). The protective effect of β1-3-D-glucan-derivatized plastic beads against *Escherichia coli* infection in mice. *Scand. J. Immunol.*, **25,** 55–60.

Sharp, J.K., McNeil, M. & Albersheim, P. (1984). The primary structures of one elicitor-active and seven elicitor-inactive hexa(β-D-glucopyranosyl)-D-glucitols isolated from the mycelial walls of *Phytophora megasperma* f. sp. glycinea. *J. Biol. Chem.*, **259,** 11 321–11 336.

Sone, Y., Kakuta, M. & Misaki, A. (1978). Isolation and characterization of polysaccharides of 'Kikurage,' fruit body of *Auricularia auricula-judae*. *Agric. Biol. Chem.*, **42,** 417–425.

Sone, Y., Okuda, R., Wada, N., Kishida, E. & Misaki, A. (1985). Structures and antitumor activities of the polysaccharides isolated from fruiting body and the growing culture of mycelium of *Ganoderma lucidum*. *Agric. Biol. Chem.*, **49,** 2641–2653.

Steadman, R., Petersen, M.M., Topley, N., Williams, D., Matthews, N., Spur, B. & Williams, J.D. (1990). Differential augmentation by recombinant human tumor necrosis factor-α of neutrophil responses to particulate zymosan and glucan. *J. Immunol.*, **144,** 2712–2718.

Suzuki, I., Hashimoto, K., Ohno, A., Tanaka, H. & Yadomaee, T. (1989). Immunomodulation by orally administered β-glucan in mice. *Int. J. Immunopharmacol.*, **11,** 761–770. (1990). *Biol. Abstr.*, **89,** 17 839.

Suzuki, I., Hashimoto, K. & Yadomae, T. (1988). The effects of a highly branched β-1,3-glucan, SSG, obtained from *Sclerotinia sclerotiorum* IFO 9395 on the growth of syngeneic tumors in mice. *J. Pharmacobio-Dyn.*, **11,** 527–532.

Suzuki, T., Ohno, N., Adachi, Y., Cirelli, A.F., Covian, J.A. & Yadomae, T. (1991*a*). Preparation and biological activities of sulfated derivatives of (1→3)-β-D-glucans. *J. Pharmacobio-Dyn.*, **14,** 256–266.

Suzuki, T., Sakurai, T., Hashimoto, K., Oikawa, S., Masuda, A., Ohsawa, M. & Yadomae, T. (1991*b*). Inhibition of experimental pulmonary metastasis of Lewis lung carcinoma by orally administered β-glucan in mice. *Chem. Pharm. Bull.*, **39,** 1606–1608.

Suzuki, T., Tanaka, H., Kinoshita, A., Oikawa, S., Osawa, M. & Yadomae, T. (1990). Effect of orally administered β-glucan in macrophage function in mice. *Int. J. Immunopharmacol.*, **12,** 675–684. (1990). *Biol. Abstr.*, **90,** 126 659.

Tabata, K., Ito, W., Kojima, T., Kawabat, S. & Misaki, A. (1981). Ultrasonic degradation of schizophyllan, an antitumor polysaccharide produced by *Schizophyllum commune* Fries. *Carbohydr. Res.*, **89,** 121–135.

Usui, T., Iwasaki, Y., Hayashi, K., Mizuno, T., Tanaka, M., Shinkai, K. & Arakawa, M. (1981). Antitumor activity of water-soluble β-D-glucan elaborated by *Ganoderma applanatum*. *Agric. Biol. Chem.*, **45,** 323–326.

Wagner, H., Stuppner, H., Schafer, W. & Zenk, M. (1988). Immunologically active polysaccharides of *Echinacea purpura* cell cultures. *Phytochemistry*, **27,** 119–126.

Whistler, R.L., Bushway, A.A., Singh, P.P., Nakahara, W. & Tokuzen, R. (1976). Noncytotoxic, antitumor polysaccharides. *Adv. Carbohydr. Chem. Biochem.*, **32,** 235–275.

Williams, D.L., McNamee, R.B., Jones, E.L., Pretus, H.A., Ensley, H.E., Browder, I.W. & Di Luzio, N.R. (1991). A method for the solubilization of a (1→3)-β-D-glucan isolated from *Saccharomyces cerevisiae*. *Carbohydr. Res.*, **219,** 203–213.

Williams, D.L., Pretus, H.A., McNamee, R.B., Jones, E.L., Ensley, H.E. & Browder, I.W. (1992). Development of a water-soluble, sulfated (1→3)-β-D-glucan biological response modifier derived from *Saccharomyces cerevisiae*. *Carbohydr. Res.*, **235,** 247–257.

Witczak, Z.J. & Whistler, R.L. (1987). Structure and antitumor activity of polysaccharides. In *Industrial Polysaccharides*, eds S.S. Stivala, V. Crescenzi & I.C.M. Dea. Gordon and Breach Science Publ., New York, pp. 157–173.

Yamada, H., Kawaguchi, N., Ohmori, T., Takeshita, Y., Taneya, S.-I. & Miyazaki, T. (1984). Structure and antitumor activity of an alkali-soluble polysaccharide from *Cordyceps ophioglossoides*. *Carbohydr. Res.*, **125,** 107–115.

Yoshioka, Y., Tabeta, R., Saitô, H., Uehara, N. & Fukuoka, F. (1985). Antitumor polysaccharides from *P. ostreatus* (Fr.) Quél.: isolation and structure of a β-glucan. *Carbohydr. Res.*, **140,** 93–100.