ORIGINAL ARTICLE

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Orally administered β-glucans enhance anti-tumor effects of monoclonal antibodies

Abstract β-Glucan primes leukocyte CR3 for enhanced cytotoxicity and synergizes with anti-tumor monoclonal antibodies (mAb). We studied readily available (1→3)-β-D-glucan using the immune deficient xenograft tumor models, and examined the relationship of its anti-tumor effect and physico-chemical properties. Established subcutaneous (s.c.) human xenografts were treated for 29 days orally with daily β-glucan by intragastric injection and mAb intravenously (i.v.) twice weekly. Control mice received either mAb alone or β-glucan alone. Tumor sizes were monitored over time. β-Glucans were studied by carbohydrate linkage analysis, and high performance size-exclusion chromatography with multiple angle laser scattering detection. Orally administered β-D-glucan greatly enhanced the anti-tumor effects of mAb against established tumors in mice. We observed this β-glucan effect irrespective of antigen (GD2, GD3, CD20, epidermal growth factor-receptor, HER-2), human tumor type (neuroblastoma, melanoma, lymphoma, epidermoid carcinoma and breast carcinoma) or tumor sites (s.c. versus systemic). This effect correlated with the molecular size of the (1→3),(1→4)-β-D-glucan. Orally administered (1→3),(1→6)-β-D-glucans also synergized with mAb, although the effect was generally less marked. Given the favorable efficacy and toxicity profile of oral β-D-glucan treatment, the role of natural products that contain β-glucan in cancer treatment as an enhancer of the effect of mAb therapy deserves further study.

Keywords ADCC · CMC · CR3 · (1→3),(1→4)-β-D-glucan

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Introduction

Evidence of the efficacy of monoclonal antibodies (mAb) against human cancer in clinical trials is increasingly evident. However, induced or administered antibodies to human tumors have not realized their fullest therapeutic potential, even when they can activate complement-mediated cytotoxicity (CMC) and antibody-dependent cell-mediated cytotoxicity (ADCC). The deposition of C3b and iC3b on tumor cells fails to stimulate phagocytosis or extracellular cytotoxicity by C3-receptor-bearing neutrophils, macrophages, and natural killer (NK) cells, even though these same effector cells can efficiently kill C3b and iC3b opsonized microorganisms. The receptor for iC3b, CR3 (also called CD11b/CD18, Mac-1, or $\alpha_M\beta_2$-integrin), is found in monocytes/macrophages, NK cells, and immune cytotoxic T lymphocytes (CTL). CR3 activation requires the engagement of two sites on its α-subunit (CD11b): the iC3b-binding site within the I-domain at the N-terminus and a lectin site at the C-terminus [41, 42]. β-Glucans are specific for the lectin site. When coated with iC3b, yeast cells (with their β-glucan-containing cell wall), engage both iC3b and lectin binding sites on leukocytes, triggering phagocytosis and respiratory burst [6, 42]. In contrast, tumor cells coated with iC3b cannot activate leukocytes because they lack the CR3-binding β-glucan [20, 29, 44, 54]. Soluble forms of β-glucans can bind to the lectin site [50, 57] and prime both phagocytic and NK cells to kill iC3b-coated tumor targets [53, 54, 57]. When this strategy was tested in mice carrying mammary tumors, where natural IgM anti-tumor antibodies deposited iC3b on tumor cell surface, intravenous (i.v.)

administration of β-glucan alone induced impressive tumor shrinkage [58]. In the absence of such natural antibodies (SCID mice), or of C3 (C3 knockout mice), or of leukocyte CR3 (CR3 knockout mice), these tumors were resistant to β-glucan therapy [58].

β-Glucans are polymers of glucose that are extractable from cereals, mushrooms, seaweed and yeasts [4]. They are (1→3)-β-D-glucopyranosyl polymers with randomly dispersed single β-D-glucopyranosyl units attached by (1→6)-β linkages, giving a comb-like structure. The (1→3)-β-backbone and the (1→6)-linked branches were thought to be important for their immune effects. Lentinan (β-glucan extracted from from the mushroom *Lentinus edodes*, Basidiomycete family) is a high molecular weight (m.w.) β-glucan with (1→6) branches off every three (1→3)-β-D-glucopyranosyl residues, and has been licensed in Japan for cancer treatment. Schizophyllan (from *Schizophyllum commune*, Basidiomycete family) and β-glucan from Baker's yeast (*Saccharomyces cerevisiae*) have similar structures. Laminarin (from seaweed), a small m.w. β-glucan, has (1→6)-β branches occurring at every ten (1→3)-β-D-glucopyranosyl units. On the other hand, β-glucan from barley, oat or wheat has mixed (1→3),(1→4)-β linkage in the backbone, but no (1→6)-β branches and is generally of high m.w.

More recently, the human β-glucan receptor, DECTIN-1, was cloned and found to be expressed on monocytes, macrophages, dendritic cells (DC), and NK cells [55]. This is a type II transmembrane receptor with a single extracellular carbohydrate recognition domain and an immunoreceptor tyrosine activation motif in its cytoplasmic tail. It functions as a pattern recognition receptor, recognizing a variety of (1→3)-β- and/or (1→6)-β-linked glucans, including mixed linkage (1→3),(1→4)-β-glucans. This receptor also binds T lymphocytes using a domain distinct from the β-glucan binding site. DECTIN-1 gene has been mapped to chromosome 12p13 within the NK gene cluster complex [46].

Oral administration of botanical and yeast extracts containing (1→3),(1→6)-β-glucans has shown anti-tumor effects in animal studies [13, 16, 33, 34, 37, 48], and in patients with colorectal cancer [52] and gastric cancer [32], although results have been less encouraging for breast cancer [18, 51] and leukemia [39]. However, none of these previous reports recognized the critical contribution from anti-tumor antibodies in orally administered β-glucan activity. Furthermore, oral β-glucans have not been rigorously tested in immune-deficient models, a mimic of real-life situation among cancer patients undergoing chemotherapy. In addition, the formulations previously explored were not chemically pure glucans, a major limitation for quality control and further clinical development. Although (1→3),(1→6)-β-glucans were the primary focus of most previous investigations, (1→3),(1→4)-β-glucans, despite their wide distribution in the daily diet, have not been rigorously tested in their purified forms.

(1→3),(1→4)-β-Glucans derived from barley have been shown to bind to CR3 in vitro [57], to activate ADCC mediated by NK cells [11, 53, 54], monocyte [10, 50], and neutrophils [50], as well as to stimulate tumor necrosis factor (TNF) production by monocytes [43]. However, their in vivo immunomodulatory effects, especially when administered by oral route, have not been tested. We have recently reported on an unusually strong synergism between anti-GD2 antibodies and intragastric injection of barley β-D-glucan against human neuroblastoma xenografts [7]. NK cells were only partly responsible for the anti-tumor effect, and the IgM anti-GD2 antibody 3G6 (class switch variant of 3F8 with no ADCC activity) was equally effective. Here we extend this observation to a diverse panel of clinically important mAb in a broad spectrum of common human cancers. We further examine the relationship between molecular size (1→3)-β-D-glucan and its synergy with mAb.

Materials and methods

Cell lines

The cell lines Daudi, SKMel-28 and A431 were obtained from the American Type Culture Collection (ATCC; Rockville, Md.). LAN-1 was provided by R. Seeger (Children's Hospital of Los Angeles, Los Angeles, Calif.); NMB7 by S.K. Liao (McMaster University, Ontario, Canada); human breast carcinoma cell line BT474 was kindly provided by D. Solit (MSKCC; Memorial Sloan-Kettering Cancer Center, N.Y.); and SKNJD and SKNER were established at MSKCC. BT474 was cultured in Dulbecco's modified Eagle's medium (DMEM) with nutrient mixture F12 (DMEM/F-12; Life Technologies, Gaithersburg, Md.) in a 1:1 mixture fortified with 10% newborn calf serum (Hyclone, Logan, Utah), MEM non-essential amino acids (Life Technologies), 100 U/ml of penicillin (Sigma, St. Louis, Mo.), and 100 µg/ml of streptomycin (Sigma). All other cell lines were cultured in RPMI 1640 (Life Technologies) containing 10% defined calf serum (Hyclone) and 100 U/ml of penicillin, 100 µg/ml of streptomycin and 2 mM L-glutamine (Sigma).

Antibodies

mAb 3F8 (mouse IgG3) and 3G6 (mouse IgM) reactive against GD2 ganglioside expressed on neuroectodermal tumors, and mAb 8H9 (mouse IgG1) reactive with a glycoprotein expressed on these same tumors, have been previously described [8, 31]. They were purified to >90% purity by affinity chromatography: protein A (Pharmacia, Piscataway, N.J.) for 3F8, and protein G (Pharmacia) for 8H9. Anti-GD3 antibody (R24) [17] was provided by P. Chapman (MSKCC). Hybridomas producing the anti-epidermal growth factor receptor (EGF-R) antibodies 528 (IgG2a) and 455 (IgG1) were obtained from ATCC [28]. Rituximab (anti-CD20) and Herceptin (anti-HER2) were purchased from Genentech (San Francisco, Calif.).

β-Glucan

Barley, oat and lichenan β-D-glucans were purchased from Sigma and Megazyme International Ireland (Wicklow, Ireland). Although endotoxin cannot prime CR3 in the same way as β-glucan [53], barley β-glucans were tested and found negative for bacterial or mycoplasma contamination and <0.1 EU/mg of endotoxin by

kinetic-chromogenic limulus amebocyte lysate (LAL) test. In addition, they were all found to contain <0.1% nitrogen and <0.0001% heavy metals by Kjeldahl analysis. Betatrim (Quaker Oatrim; 5% β-glucan from oats) was provided by Rhodia Food (Cranbury, N.J.). Lentinan was provided by the Drug Synthesis and Chemistry Branch (Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, Bethesda, Md.). β-Glucan was dissolved completely by boiling for 10 min in normal saline, remained in solution at room temperature and freely passed through a 0.2-μm Millipore filter. Lentinan was dissolved first in DMSO before diluting in water. Digestion with Lichenase (endo-1,3:1,4-β-D-glucanase) from *B. subtilis* (Megazyme) was carried out in sodium phosphate buffer (20 mM; pH 6.5) at 40°C for 10 min. Sugar composition and linkage analysis by gas chromatography-mass spectrometry following methylation was performed by the Complex Carbohydrate Research Center [University of Georgia, Athens, Ga.; supported in part by the Department of Energy-funded Center for Plant and Microbial Complex Carbohydrates (DF-FG09–93ER-20097)] [9]. The average ratio of (1→3) to (1→4)-β-linkage in (1→3),(1→4)-β-D-glucans derived from barley, oat and wheat was 3:7. For molecular size and shape estimations, β-glucan was analyzed by size-exclusion chromatography plus multiple-angle laser light scattering (MALLS), as previously described [25, 56]. Besides measuring molecular size by MALLS, the slope derived from root mean square radius versus molar-mass plots gave an estimate of the molecular shape: a slope of 0.33 being the shape of a sphere, 0.5 being random coils and 1.0 being rigid rods. The shape of high m.w. β-glucans tended to be more that of random coils in contrast to low m.w. species which were more sphere-like.

Mice and treatment

We chose the mouse model because of its relative inefficiency in CDC and ADCC [2], and mAb alone were typically ineffective against established tumors. Athymic nu/nu mice were purchased from the National Cancer Institute-Frederick Cancer Center (Bethesda, Md.) and ICR/SCID from Taconic (White Plains, N.Y.) and maintained in ventilated cages. Experiments were carried out under Institutional Animal Care and Use Committee (IACUC) approved protocols, and institutional guidelines for the proper and humane use of animals in research were followed. Experiments were carried out at least twice. Tumor cells were planted ($1–5 \times 10^6$ cells) s.c. in 100 μl of Matrigel (Sigma). Tumor dimensions were measured 2 to 3 times a week with vernier calipers, and tumor size was calculated as the product of the 2 largest perpendicular diameters. For breast carcinoma xenograft studies, 6–8-week old female nude mice (NCI) were initially implanted with 0.72 mg 90-day release 17β-estradiol pellet (Innovative Research of America, Sarasota, Fl.) s.c. into the right flank. Twenty-four hours later, 10^7 BT474 cells were implanted s.c. into the left flank. All treatment studies started in groups of 4–5 mice when tumor diameters reached 0.7 to 0.8 cm. Mice received antibody treatment (40–200 μg per day; i.v., by retroorbital injection) twice weekly and oral β-glucan (400 μg per day) by intragastric injection every day for a total 4 weeks. Mice were weighed once a week and killed according to IACUC guidelines. In the SCID mouse systemic human lymphoma (Daudi) model, 5 million cells were administered i.v., and treatment started 10 days later.

Statistical analysis

Because measurement times varied between experiments, and mice in the control groups were frequently killed (as required by IACUC for rapidly enlarging tumors) before the end of each experiment, tumor growth was calculated by fitting a regression slope for each individual mouse to log-transformed values of tumor size. Slopes were compared between groups using linear regression with mAb treatment, β-glucan treatment and combination treatment as covariates. In the study of melanoma tumor growth, β-glucan had an anti-tumor effect at both 400 μg and 40 μg dose levels; dose was added as a covariate for analysis. In the study of epidermoid tumor growth, β-glucan had anti-tumor effect at all 3 mAb doses tested (200 μg, 40 μg, 10 μg); antibody dose was added as a covariate. Trends for slope by molecular weight were tested by regression analysis of slope scores. Survival analysis was conducted by Cox regression using the indicator variables: mAb treatment, β-glucan treatment, and combination treatment; in the survival study of lymphoma, Rituximab was given at 2 different doses, and so dose of antibody was added as a covariate for analysis. All analysis were conducted using STATA (Stata, College Station, Tex.).

Results

Synergy between mAb and barley β-glucan in human tumor xenografts

We previously reported that a combination of oral administration of β-glucan (average m.w. 206 kDa) from barley alone at 400 μg q.d. × 4 weeks plus antibody 3F8 i.v. significantly inhibited neuroblastoma (LAN-1, NMB7, SK-N-JD, and SK-N-ER) tumor growth and prolonged mice survival, in contrast to either β-glucan or 3F8 alone [7]. Here, we screened a series of mAb against a panel of human tumor xenografts. The combination of oral β-glucan with complement-activating mAb suppressed tumor growth significantly in contrast to mAb or β-glucan alone. This was shown for anti-GD3 mAb (R24) against melanoma (Fig. 1A), anti-EGF-R (528) mAb against epidermoid carcinoma A431 (Fig. 1B), and anti-HER2 (Herceptin) against human breast carcinoma BT474 xenografts in nude mice (Fig. 1C). These experiments were performed 2, 3 and 3 times, respectively, with similar results. mAb 455 is an anti-EGF-R IgG1 which does not activate complement. It was ineffective against epidermoid carcinoma, in contrast to the complement-fixing IgG2a mAb 528 (Fig. 1B). Subcutaneous Daudi lymphoma was significantly suppressed when Rituximab (anti-CD20) was administered in conjunction with oral β-glucan (data not shown). In a metastatic lymphoma model, Daudi cells injected i.v. established widespread tumors in brain, spinal cord, kidneys and ovaries of SCID mice. In the Cox model, only combination treatment and dose of Rituximab were associated with survival. Median survival was 59 days in animals receiving either Rituximab alone, β-glucan alone or no treatment. Median survival in the group treated with Rituximab plus β-glucan was 97 days (hazard ratio compared to other treatments combined 0.09; 95% CI 0.03, 0.27; $P < 0.001$).

Molecular size of (1→3),(1→4)-β-D-glucan and its influence on anti-tumor effect

Barley β-glucans of increasing molecular sizes (45 kDa, 149 kDa, 207 kDa, 284 kDa, 404 kDa) were tested at an oral dose of 40 μg or 400 μg per day (daily for a total of 4 weeks) in combination with i.v. 3F8 (200 μg twice a week × 4 weeks) in mice xenografted with human neuroblastoma LAN-1. Tumor growth rate was calculated
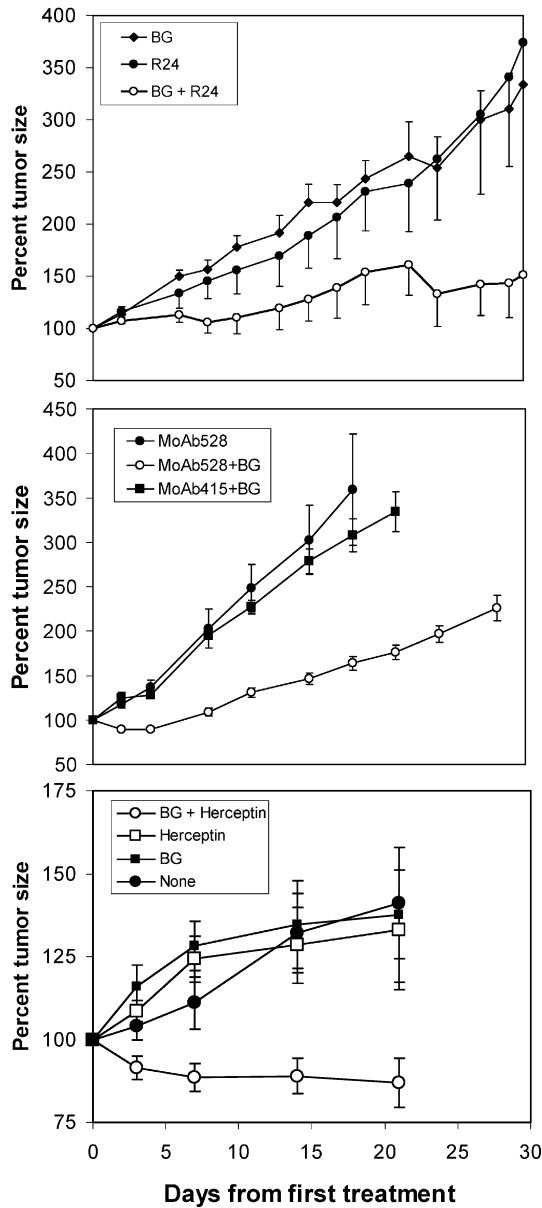
Fig. 1. Synergy of orally administered barley β-glucan with (**A**) R24 (anti-GD3) antibody against SKMel28 melanoma xenografts in nude mice. Standard barley beta-glucan of 206 kDa was used throughout. In contrast to β-glucan control (*solid diamonds*), and R24 control (*solid circles*), the combination of R24 and β-glucan (*open circles*) significantly suppressed tumor growth (tumor growth rate reduced for combination treatment by 1.2%, 95% CI 0.1%, 2.5%; *P* = 0.06); with (**B**) 528 (anti-EGF-R) mAb against epidermoid carcinoma A431 xenografts in nude mice. In contrast to β-glucan + 455 (IgG1 noncomplement fixing) control (*solid squares*), and 528 mAb alone (*solid circles*), the combination of 528 mAb and β-glucan (*open circles*) significantly suppressed tumor growth (tumor growth rate reduced for combination treatment by 1.4%, 95% CI 0.7%, 3.5%; *P* = 0.17); with (**C**) Herceptin (anti-HER2) antibody against human breast carcinoma BT474 xenografts in nude mice. In contrast to control (*n* = 4; *solid circles*), Herceptin (*n* = 9; *open squares*), or β-glucan control (*n* = 7; *solid squares*), the combination of Herceptin and β-glucan (*n* = 12; *open circles*) significantly suppressed tumor growth (tumor growth rate reduced for combination treatment by 1.9%, 95% CI 0.7%, 3%; *P* = 0.002)

for individual tumors in each treatment group and expressed as a percent of that in the saline control. When normalized to the range between minimally suppressed (45 kDa) and maximally suppressed (404 kDa), suppression of tumor growth was highly correlated with molecular size for both 40 μg and 400 μg β-glucan doses (*P* < 0.001; Fig. 2). It should be noted that the effect of size on potency may be indirect, since the shape of the β-glucan in aqueous solution also correlated with average m.w. by MALLS analysis.

Source of β-D-glucan and anti-tumor synergy with mAb

Barley (1→3),(1→4)-β-D-glucan with an average m.w. of 206 kDa was chosen as our reference standard. Using the LAN-1 neuroblastoma xenograft model, equivalent microgram doses of β-glucans derived from various plant sources were compared for their anti-tumor activity when administered by intragastric injection plus i.v. mAb 3F8 (Table 1). As expected, since the chemical composition of (1→3),(1→4)-β-D-glucan derived from barley and oats was similar, comparable levels of synergy with mAb were found, and high m.w. β-glucan was again more effective. Lentinan with (1→3),(1→6)-β-linkages (1,500 kDa) also showed activity, although less effective than barley β-glucan.

Discussion

We have shown that β-glucans greatly enhanced the anti-tumor effects of mAb against established tumors in
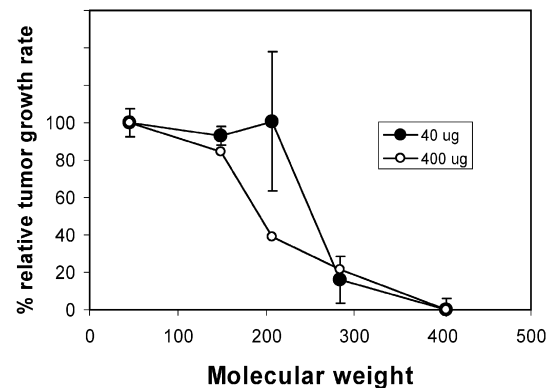

Fig. 2. Glucan size and relative tumor growth rate. Orally administered barley β-glucan of increasing molecular size was tested at 40 μg (*solid circles*) and 400 μg (*open circles*) daily for 4 weeks in combination with anti-GD2 antibody 3F8 (200 μg twice weekly for 4 weeks) against established neuroblastoma LAN-1 xenografts. Tumor growth rate was calculated for individual tumors in each treatment group and expressed as a percentage of that in the saline control, and normalized to a range between minimally suppressed (45 kDa) and maximally suppressed (404 kDa). A molecular size dependence was seen at both 40 μg and 400 μg dose levels (*P* < 0.001). Only SE for the 40 μg dose group has been shown

Table 1. Anti-tumor effect of orally administered β-glucans when used in combination with i.v. 3F8

β-Glucans	Description	Molecular weight (kDa)	Anti-tumor effect (%)[b]
$(1\rightarrow3),(1\rightarrow4)$-$\beta$-D-glucan[a]			
Standard barley glucan	–	206	100
Barley	High viscosity	321	40
	Medium viscosity	178	92
	Low viscosity	102	< 5
Oats	High viscosity	245	63
	Medium viscosity	187	44
Betatrim	–	–	45
Lichenan	–	121	56
$(1\rightarrow3),(1\rightarrow6)$-$\beta$-D-glucan			
Lentinan		1,490	47

[a]$(1\rightarrow3)$-linkage was $\sim$30% for all the $(1\rightarrow3),(1\rightarrow4)$-$\beta$-D-glucans; see Materials and methods
[b]Either 400 or 40 µg of $(1\rightarrow3)$-β-D-glucans was administered orally q.d. $\times$ 4 weeks plus i.v. mAb 3F8 twice a week (M, Th) $\times$ 8 doses to groups consisting of of 4–12 mice each. Tumor size was measured periodically over the entire treatment period. Tumor growth curve was calculated as detailed in Materials and methods, and expressed as percentage of control (treated with mAb 3F8 alone). Anti-tumor effect was normalized to that of standard barley β-glucan of m.w. 206 kDa

mice. We observed this effect irrespective of the route of β-glucan administration (intragastric or intraperitoneal), antigen (GD2, GD3, CD20, EGFR, HER2), human tumor type (NB, melanoma, epidermoid carcinoma, lymphoma, breast cancer), mouse strain (athymic nu/nu, severe combined immune deficiency mice), or tumor site (s.c. versus systemic). β-Glucan was heat-stable, its anti-tumor effect was dose- and schedule-dependent, requiring antibody-Fc, but not cytophilicity of the antibody. Neither antibody nor β-glucan alone was effective. This synergy of $(1\rightarrow3),(1\rightarrow4)$-$\beta$-D-glucan with mAb increased with β-glucan m.w. This is the first report demonstrating the importance of molecular size in orally administered $(1\rightarrow3),(1\rightarrow4)$-$\beta$-D-glucan. These findings were not entirely intuitive, since small molecules (often thought to be easier to process or absorb) were less effective in our tumor model. More importantly, we extended our initial observation of the effect of oral administration of barley β-glucan on antibody function in neuroblastoma [7] to a broad variety of clinically relevant mAb in well established, clinically relevant xenograft models. These findings laid to rest our concern that the anti-tumor effect when barley β-glucan was combined with anti-disialoganglioside antibody was a consequence of direct or indirect interaction of β-glucan with carbohydrate tumor antigen or epitopes (e.g. GD2), or a unique interaction on neuroblastoma tumors.

β-Glucans have been tested for tumor therapy in mice for nearly 40 years [12, 49]. Several forms of mushroom-derived β-glucans are used clinically in Japan to treat cancer, including polysaccharide Kureha (PSK; from *Coriolus versicolor*), Lentinan and Schizophyllan. In Japan, PSK and Schizophyllan were shown to improve survival rates in some cancer trials [14, 30, 35, 36, 4040], but with less encouraging results in others [38, 51]. While β-glucans are not used by western oncologists, β-glucan containing botanical medicines such as Ling-Zhi, maitake and green barley are widely used to treat cancer patients in the US as alternative/complementary cancer therapies, often with insufficient clinical validation or quality control.

Given the biology of iC3b targeted cytotoxicity, β-glucan should have clinical potential. However, there are several limitations to existing β-glucan strategies. They are generally expensive and inconvenient to administer: e.g. Lentinan and Schizophyllan are given i.v. daily over long periods of time. Besides being insoluble, they contain proteins and non-β-glucan carbohydrates, which confound mechanistic studies and complicate the manufacturing and control process. Because of protein contaminants they are potentially allergenic. The spontaneous cross-linking of CR3 by β-glucan of high m.w. can cause neutrophil degranulation and cytokine release from macrophages, resulting in undesirable clinical toxicities. Regarding low m.w. β-glucans, besides their low affinity for CR3, they have rapid renal clearance. Without anti-tumor antibodies to activate human complement, β-glucan is largely ineffective. In this report we addressed these limitations by: (1) using β-glucan from readily available sources, e.g. $(1\rightarrow3),(1\rightarrow4)$-$\beta$-D-glucan from barley; (2) administering β-glucan orally instead of intravenously; and (3) including the coadministration of tumor-specific antibodies to ensure complement activation.

Previous studies have demonstrated that orally administered β-glucans activate splenic and peritoneal macrophages for tumor cytotoxicity. In a study of ^{14}C-labeled orally administered β-glucan, sequestration in the liver was observed [19], suggesting that it entered the blood and behaved pharmacokinetically in a similar manner to i.v. administered low m.w. β-glucan [45, 47, 59]. These studies also suggested that processing by the gastrointestinal tract produced β-glucan with high activity for CR3. Besides this model of intravasation of processed barley β-glucan, leukocytes could also be activated directly in the gut before homing to the tumor. Despite the abundance of β-glucan (3% of the dry weight) in grains, its bioavailability from cereals is limited since high m.w. β-glucans require high temperature ($>60^{\circ}$C) for solubilization and gelling. It is of interest that unpurified β-glucan (Betatrim) has low solubility and poor gelling properties, and has low biologic activity

in our model. Recent studies using high-fiber (13.5 g/day) wheat-bran supplement [1] did not show anti-tumor effect in human trials. Besides the issue of bioavailability, the effectiveness of glucans or other agents such as wheat bran cannot be assessed in the absence of mAb administration, since our studies clearly showed that even the most effective β-glucan required the simultaneous administration of mAb. Previous studies that investigated the therapeutic effect of β-glucan administration did not incorporate co-administration of mAb as part of the protocol. It is likely that when β-glucan functions without co-administration of mAb, its tumor cytotoxic effect requires the presence of naturally-occurring antitumor antibodies that target the tumor with iC3b.

Our findings using (1→3),(1→4)-β-D-glucan from barley were unexpected. In previous studies, (1→3), (1→6)-β linkage (e.g. Lentinan, Schizophyllan, Laminarin, and β-glucan from Baker's yeast) was deemed requisite for its anti-tumor effect [4]. In those models, however, T cells were essential. This T cell requirement was later shown to involve helper T cell function needed for the generation of naturally-occurring antitumor antibodies. Thus, the therapy was shown to be effective in T cell deficient SCID or nude mice given natural antibodies isolated from normal mouse serum [58]. In our studies, while (1→3),(1→4)-β-D-glucan alone was completely inactive, it enhanced the anti-tumor effect of mAb in immunodeficient mice, supporting the model in which β-glucans function in tumor therapy by binding to CR3 in vitro to mediate leukocyte CR3-dependent cytotoxicity, and that neither T nor B cells are needed. Since most cancers express mCRP (CD46, CD55, CD59) on their cell surface [3, 5, 15, 21, 22, 23, 26, 27], complement-mediated tumor lysis is typically inefficient. Nevertheless, despite these inhibitory proteins, iC3b has been detected on tumor cells isolated from fresh human breast tumors, and enough levels could be deposited by antibodies in vitro to opsonize tumor cells for phagocytes and NK cells in vitro [24]. It is possible that sublethal levels of complement activation deposited enough iC3b to optimize tumor killing, a strategy that can be enhanced by oral administration of β-glucans.

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