Oral (1→3),(1→4)-β-D-Glucan Synergizes with Antiganglioside GD2 Monoclonal Antibody 3F8 in the Therapy of Neuroblastoma[1]

Nai-Kong V. Cheung[2] and Shakeel Modak

Department of Pediatrics, Memorial Sloan-Kettering Cancer Center, New York, New York 10021

ABSTRACT

Purpose: In vitro β-glucan can enhance tumor cytotoxicity through iC3b receptors on leukocytes. We tested if (1→3),(1→4)-β-D-glucan (β-glucan) can synergize with anti-GD2 monoclonal antibody (MoAb) 3F8 (mouse IgG3) in therapy of human neuroblastoma xenografts.

Experimental Design: Athymic nude mice with established neuroblastoma xenografts were treated with daily i.p. or p.o. β-glucan, in the presence/absence of i.v. MoAb twice a week, for 22–29 days. Serial tumor volumes and body weights were monitored.

Results: 3F8 plus β-glucan produced near-complete tumor regression/disease stabilization, whereas 3F8 or β-glucan alone did not significantly affect tumor growth. For NMB7 tumors, median survival of 3F8 plus β-glucan group was 5.5-fold that of control groups ($P < 0.001$), and for LAN-1, the survival difference was 2.6-fold. Forty-seven percent of the mice with NMB7 and 18% with LAN-1 remained progression free in contrast to <3% of controls. Antitumor effect was seen at ≥40 µg of glucan dose, i.v. or p.o., and in all human neuroblastoma cell lines tested. No toxicities were noted in mice treated with either β-glucan alone or 3F8 plus β-glucan (4–4000 µg/dose). In contrast to anti-GD2 MoAb 3G6 (IgM), 3F8 F(ab′)₂ and MoAb 8H9 (IgG1) did not activate complement and had no synergy with β-glucan. Antitumor effect of 3F8 plus p.o. β-glucan persisted after antiasialo-GM1 antibody treatment, as well as in NK-deficient host.

Conclusions: p.o. 1,3–1,4-β-glucan synergized with antitumor IgG and IgM MoAb *in vivo*. Because β-glucan was well tolerated and inexpensive, its potential value in cancer therapy deserves further investigation.

INTRODUCTION

MoAb[3] selective for tumors have therapeutic potential (1). The introduction of hybridoma technology by Koehler and Milstein (2) in 1975 and advances in molecular biological techniques have greatly expanded the potential of MoAb in human cancers. Evidence of efficacy in clinical trials is increasingly evident: 17-1A in colon cancers (3), anti-CD20 in lymphoma (4, 5), anti-HER2 antibodies in breast cancer (6, 7), and M195 against CD33 in acute leukemia (8) are good examples. Our laboratory has developed the MoAb 3F8, which targets the ganglioside GD2 overexpressed on neuroblastoma. 3F8 has been shown to have high specificity and sensitivity in the radioimmunodetection of minimal residual disease in patients with NB (9) and a significant clinical impact when used as adjuvant therapy (10).

The immune basis of clinical tumor response to MoAb includes direct cytotoxicity, and induced immunity. ADCC and complement-mediated cytotoxicity are responsible for the direct killing of tumor cells. On the other hand, through tumor opsonization (11) or idiotype network (12), tumor-specific immunity is induced. β-glucans are polymers of glucose extractable from cereals, mushrooms, seaweed, and yeasts (13). They are (1→3)-β-D-glucopyranosyl polymers with randomly dispersed single β-D-glucopyranosyl units attached by (1→6)-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 (from Lentinus edodes, Basidiomycete family) is a high MW β-glucan with (1→6) branches off every three (1→3)-β-D-glucopyranosyl residues, and it 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 MW β-glucan, has (1→6)-β branches occurring at every 10 (1→3)-β-D-glucopyranosyl units. On the other hand, β-glucan from barley, oat, or wheat has mixed (1→3)- and (1→4)-β-linkage in the backbone, but no (1→6)-β branches, which are generally of high MW. Although barley (1→3),(1→4)-β-D-glucan has been shown *in vitro* to bind to CR3 (14) and activate ADCC mediated by NK cells (15–17), monocytes (18, 19), and neutrophils (17, 19), as well as stimulating TNFα production by monocytes (20), their *in vivo* immunomodulatory effects in cancer models have yet to be investigated.

We now report our findings that p.o. (1→3),(1→4)-β-D-glucan derived from barley or oats can greatly enhance the activity of antitumor monoclonal antibodies in xenograft mod-
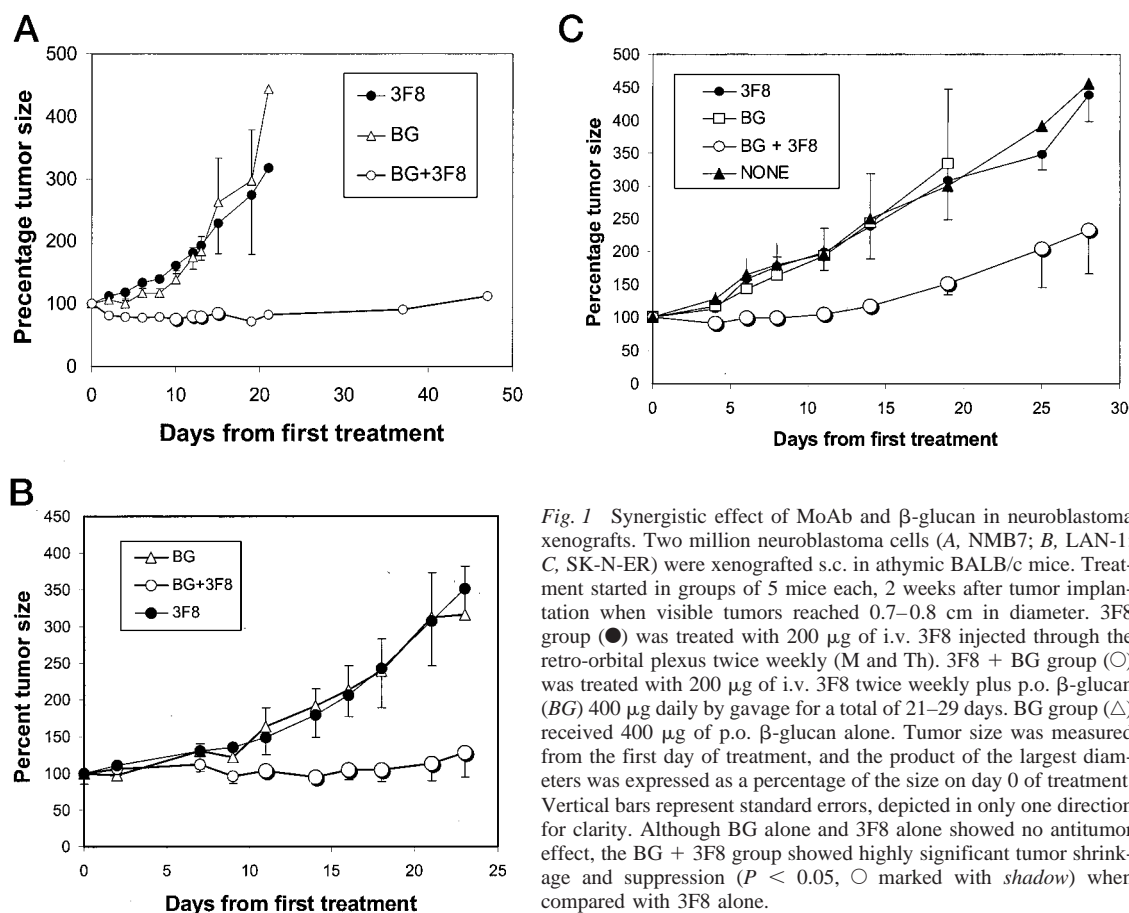
Fig. 1 Synergistic effect of MoAb and β-glucan in neuroblastoma xenografts. Two million neuroblastoma cells (*A,* NMB7; *B,* LAN-1; *C,* SK-N-ER) were xenografted s.c. in athymic BALB/c mice. Treatment started in groups of 5 mice each, 2 weeks after tumor implantation when visible tumors reached 0.7–0.8 cm in diameter. 3F8 group (●) was treated with 200 μg of i.v. 3F8 injected through the retro-orbital plexus twice weekly (M and Th). 3F8 + BG group (○) was treated with 200 μg of i.v. 3F8 twice weekly plus p.o. β-glucan (*BG*) 400 μg daily by gavage for a total of 21–29 days. BG group (△) received 400 μg of p.o. β-glucan alone. Tumor size was measured from the first day of treatment, and the product of the largest diameters was expressed as a percentage of the size on day 0 of treatment. Vertical bars represent standard errors, depicted in only one direction for clarity. Although BG alone and 3F8 alone showed no antitumor effect, the BG + 3F8 group showed highly significant tumor shrinkage and suppression (*P* < 0.05, ○ marked with *shadow*) when compared with 3F8 alone.

els. Because β-glucan is nontoxic, well tolerated, and inexpensive, its role in cancer therapy deserves careful study.

MATERIALS AND METHODS

Cell Lines. Human neuroblastoma cell line LAN-1 was provided by Dr. Robert Seeger, Children's Hospital of Los Angeles, Los Angeles, CA, and NMB7 by Dr. Shuen-Kuei Liao (McMaster University, Ontario, Canada). Neuroblastoma cell lines SK-N-JD, SK-N-ER, and SK-N-MM were established from patients with metastatic disease treated at Memorial Sloan-Kettering Cancer Center, New York, NY. Cell lines were cultured in 10% defined calf serum (Hyclone, Logan, UT) in RPMI with 2 mM L-glutamine, 100 units/ml penicillin (Sigma Chemical Co., St. Louis, MO), and 100 μg/ml streptomycin (Sigma Chemical Co.), 5% CO_2 in a 37°C humidified incubator. Normal human mononuclear cells were prepared from heparinized bone marrow samples by centrifugation across a Ficoll density separation gradient.

Antibodies. Monoclonal antibodies 3F8 (mouse IgG3), 3G6 (mouse IgM), and 8H9 (mouse IgG1) reactive with neuroblastoma have been described previously (21, 22). They were produced as ascites and purified by affinity chromatography: protein A (Pharmacia, Piscataway, NJ) for 3F8 (21), protein G (Pharmacia) for 8H9 (22), and C1q-Sepharose (Pierce, Rockford, IL) for 3G6 (21, 23). These antibodies were >90% pure by

SDS-PAGE. F(ab′)2 fragments were prepared by pepsin digestion as reported previously (24). TIB114 (N.S.7), a hybridoma secreting an IgG3 control antibody, was obtained from American Type Culture Collection. Rabbit antiasialo-GM1 antibody (Wako Pure Chemical Industries, Osaka, Japan) diluted to 1 mg/ml protein was administered at 200 μl i.p. on days 0, 1, 2, 7, 14, and 21.

Glucan. (1→3),(1→4)-β-D-glucan derived from barley and (1→4)-β-D-mannan were purchased from Sigma Chemical Co. Sugar composition and linkage analysis were 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). Barley glucan was dissolved by boiling for 10 min in normal saline.

Mice and Treatment. Athymic BALB/c mice were purchased from National Cancer Institute, Frederick, MD, and CB-17 SCID-Beige mice from Taconic (Germantown, NY). Tumor cells were planted (1–5 × 10^6 cells) in 100 μl of Matrigel (BD BioSciences, Bedford, MA) s.c. Experiments were carried out under Institutional Animal Care and Use Committee-approved protocols, and institutional guidelines for the proper and humane use of animals in research were followed. After implantation, tumor sizes (maximum width and lengths) were measured. Tumor size was calculated as a product of the

two perpendicular diameters. Treatment studies started in groups of 4–5 mice/cage when tumor diameter reached 0.7–0.8 cm, usually by 14–21 days of tumor implantation. Mice received antibody treatment i.v. (generally 200 μg/dose by retroorbital injection) twice weekly and β-glucan by intragastric gavage (generally 400 μg/dose) every day for 3 weeks (22 days of β-glucan and six doses of antibody). Mice were weighed once a week, and tumor size was measured twice a week. Mice were sacrificed when tumors reached sizes that interfered with their wellbeing.

Assays for Soluble Cytokines. Sera from mice were obtained 1, 4, 8, 24, 48, and 72 h after p.o. β-glucan. They were assayed for soluble cytokine IL-12 (p70) and TNFα, all reagents from Endogen (Woburn, MA). Briefly, 96-well microtiter plates were coated with either monoclonal antimouse IL-12 at 5 μg/ml or monoclonal anti-TNFα at 0.8 μg/ml overnight at ambient temperature. The mouse IL-12 standard ranged from 1000 pg/ml in 1:3 serial dilutions, and the TNFα standard ranged from 490 pg/ml in 1:2 serial dilution. Test samples (serum diluted 1:2) were added to the plates and incubated for 2 h at ambient temperature. The detecting antibody, biotinylated antimouse IL-12 monoclonal at 1:100 dilution for the IL-12 assay, or biotinylated antimouse TNFα monoclonal at 1:50 for the TNFα ELISA was added. The plates were incubated at ambient temperature for 1 h. After PBS wash, the secondary antibody, which was horseradish peroxidase-conjugated streptavidin at 1:400 for IL-12, and 1:200 for TNFα, was added to the plates for a 30-min incubation at ambient temperature. After another wash, tetramethylbenzidine was added as the substrate for the color reaction for 30 min, and absorbance was read at 450 nm using an ELISA plate reader. The limits of detection were 12 pg/ml for the mouse IL-12 ELISA and 10 pg/ml for the mouse TNFα ELISA.

Immunostaining for Tumor Vasculature. LAN-1 xenografts were removed 1, 4, 8, 16, 24, 48, 96, and 216 h after treatment. Tumor vasculature was assayed by immunostaining with an antiblood vessel antibody. Cryostat frozen tumor sections (at 8 mm each) were fixed in acetone and washed in PBS. Endogenous peroxidases were blocked in 0.3% H_2O_2 in PBS. Sections were incubated in 3% BSA containing 0.25% gelatin for 60 min, after the avid-biotin blocking step. Incubation with the biotinylated rat antimurine platelet/endothelial cell adhesion molecule IgG2a MoAb, MEC13.3 (1 mg/ml; BD PharMingen, San Diego, CA), was carried out at room temperature for 60 min followed by avidin-biotin complex (Vector Laboratories, Burlingame, CA). Color was developed with 3,3'-diaminobenzidine peroxidase substrate kit (Vector Laboratories). A 10% hematoxylin counterstain for 4 min was used.

Statistical Analysis. Average tumor size over time between groups was compared and tested for significant difference ($P < 0.05$) by Student's t test.

RESULTS

Synergy between Barley β-Glucan and Anti-GD2 Antibody 3F8 in Eradicating Human Neuroblastoma. 3F8 is a murine IgG3 MoAb that activates mouse and human complement and mediates effective ADCC against human neuroblastoma cells *in vitro*. β-glucan when administered p.o. at 400
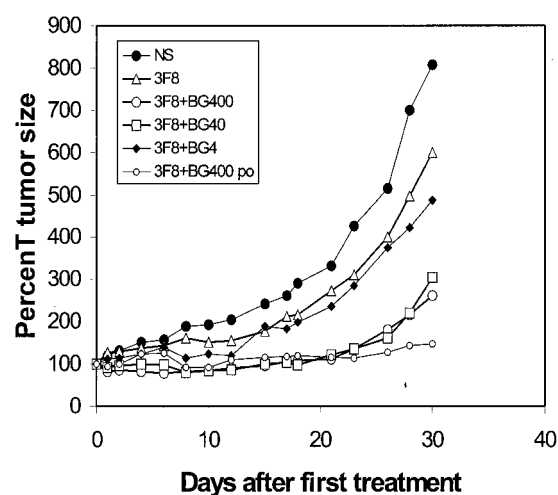

Fig. 2 Dose response of i.p. β-glucan. Two million NMB7 xenografted athymic nude mice were treated at the time of visible tumors with 3F8 alone, normal saline control, or 3F8 plus increasing doses of i.p. BG [4 μg (), 40 μg (□), and 400 μg (large ○)] or 400 μg of p.o. BG (small ○). Highly significant tumor shrinkage and suppression was shown in the combination groups except at 4 μg of BG dose. p.o. BG appeared to be more effective than i.p. BG.

μg/day had no appreciable effect on NMB7 tumor growth as did antibody 3F8 given i.v. alone. However, when β-glucan and 3F8 were used in combination, tumor growth was near totally suppressed. In >47% of mice, tumors remained permanently suppressed after treatment. Similar observations were made with neuroblastoma cell lines derived from different sources: LAN-1 (Fig. 1*A*), NMB7 (Fig. 1*B*), SK-N-ER (Fig. 1*C*), SK-N-MM, and SK-N-JD (data not shown). β-glucan was equally effective when administered p.o. or i.p. In contrast, for the GD2-negative rhabdomyosarcoma, HTB82, 3F8 plus β-glucan treatment was ineffective (data not shown). In addition, TIB114 (IgG3 control) plus barley β-glucan, or 3F8 plus mannan, had no antitumor (data not shown). When 3F8 dose was decreased from 200 to 40 μg, the antitumor effect was lost (data not shown). There was no detectable serum IL-12 or TNF-α release after p.o. β-glucan administration (data not shown). There was no immunohistochemically detectable effect of β-glucan on tumor vessel formation (data not shown).

Dose-Response Curve for i.p. β-Glucan. When the dose of i.p. β-glucan was decreased by 10-fold from 4000 μg, it was clear that 4 μg was no longer effective in synergizing with MoAb 3F8 in suppressing NMB7 growth. Interestingly, both i.p. and p.o. 1,3–1,4-β-glucan (at 400 μg/day) were effective (Fig. 2).

p.o. β-Glucan *versus* i.p. β-Glucan. When p.o. β-glucan was studied in NMB7 tumors (Fig. 3), similar dose response was found. Although an p.o. dose of 400 μg was curative for some tumors, breakthroughs were seen for lower dose levels, with those receiving 4 μg escaping sooner than those receiving 40 μg. Using the LAN-1 tumor model, neither 4 nor 40 μg of β-glucan were effective (data not shown). There was no significant body weight change in any of the treatment groups (after accounting for tumor weight), irrespective of
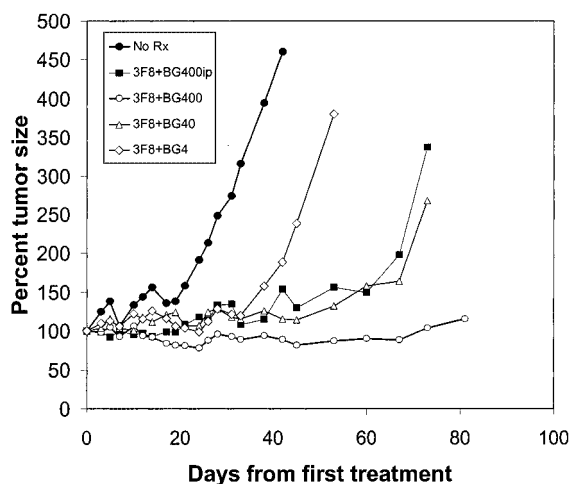
Fig. 3 Dose response of p.o. β-glucan. NMB7 xenografted in nude mice was treated as in Fig. 3 except that the dose response of p.o. β-glucan [4 μg (◇), 40 μg (△), and 400 μg (○)] was compared with 400 μg of i.p. BG (■). Control group received saline (●). A dose of 400 μg p.o. was again highly significant in eradicating or suppressing tumor growth. A dose of 400 μg i.p. appeared to be as effective as 40 μg p.o. A dose of 4 μg was the least effective.

β-glucan dose or coadministration with 3F8. At necropsy on day 22, there were no appreciable differences in the peripheral blood counts, cholesterol, and blood chemistry between mice receiving different β-glucan doses. There was also no difference in the histological appearances of organs in mice treated with β-glucan at any of the dose levels, when compared with control mice that received saline.

By the p.o. Route, Daily β-Glucan Schedule Was Necessary. A 5 days/week p.o. β-glucan regimen was comparable to the daily regimen. In contrast, a once a week or twice a week schedule of β-glucan had no antitumor effect (data not shown).

Role of NK Cells in β-Glucan Effect. Removal of NK cells by anti-Asialo GM1 antiserum eliminated a substantial amount, although not completely the β-glucan effect (Fig. 4A). Antitumor activity of β-glucan plus 3F8 was significantly decreased in the presence of anti-NK antiserum. Moreover, in SCID-beige mice, which lacked NK cells, β-glucan plus 3F8 was significantly more effective than 3F8 alone (Fig. 4B), suggesting that at least part of the antitumor activity was mediated by NK-independent cytotoxicity.

IgG3-F(ab′)2 or IgG1 Antibodies Did Not Have Antitumor Activity. The role of Fc in mediating the antitumor effect of β-glucan was apparent when Fc was removed by pepsin or when IgG1 isotype MoAb 8H9 was used (data not shown). Neither was able to activate complement or mediate efficient ADCC, and neither has significant antitumor effect when administered with 400 μg of p.o. β-glucan.

Synergy of β-Glucan with 3F8 in Prolonging Survival. Nude mice (*n* = 22) with established neuroblastoma NMB7 xenografts (0.7–0.8-cm diameter tumor at the beginning of treatment) were treated with 3F8 (200 μg twice a week i.v.) and 400 μg of β-glucan p.o. daily for a total of 3 weeks. Control mice received either saline alone (*n* = 10), 3F8 alone (*n* = 8),
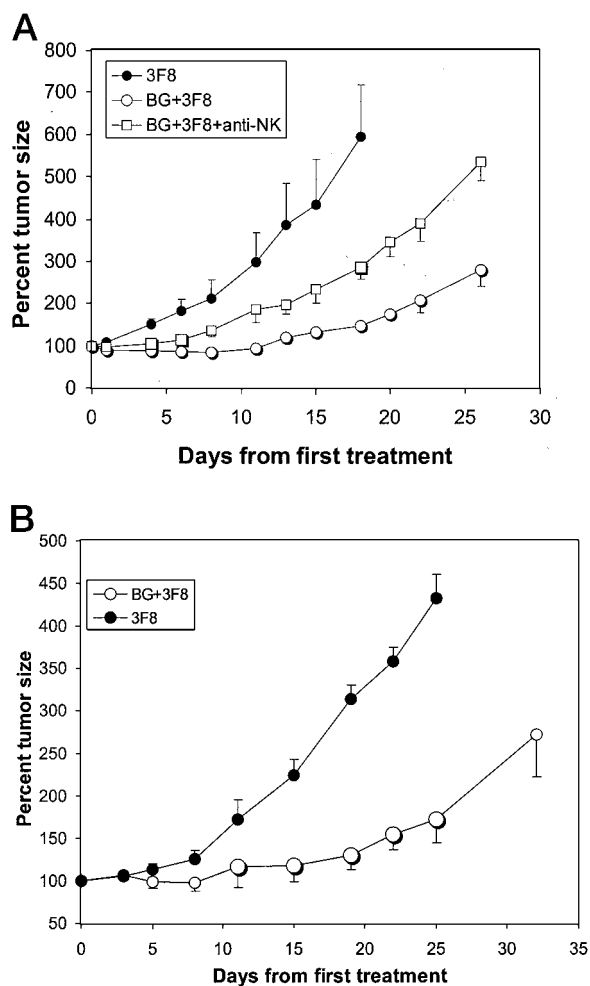
Fig. 4 A, removal of NK cells by anti-Asialo GM1 antiserum decreased but did not eliminate the antitumor effect of β-glucan plus 3F8 on LAN-1 xenografts. When compared with 3F8 control, tumor growth in BG + 3F8 group was significantly suppressed throughout (*P* < 0.05, ○ with *shadows*), whereas in the BG + 3F8 + anti-NK group (□), only on days 4, 6, and 18 (*P* < 0.05, □ with *shadows*). Anti-NK treatment was significant in reducing antitumor effect of BG + 3F8 (*P* < 0.05, days 6–26). *B,* β-glucan effect on LAN-1 xenografts in CB-17 SCID-Beige mice. Significant differences in tumor sizes were seen between 3F8 + β-glucan group (○) and 3F8 alone group (●), from days 11–25 (*P* < 0.05, ○ with *shadows*). For more details, see legend of Fig. 1.

or β-glucan alone (*n* = 16). Median survival was 30 days in control groups and 166 days in the treatment (3F8 plus β-glucan, *n* = 22) group (*P* < 0.001). Long-term survival was estimated at 47% in the treatment group and 3% in the control group (saline alone, 3F8 alone, or β-glucan alone; Fig. 5). Similar experiments were carried out in nude mice bearing established LAN-1 xenografts (also 0.7–0.8-cm diameter tumor at the beginning of treatment). Among control mice treated with either saline alone (*n* = 31), 3F8 alone (*n* = 16), or β-glucan alone (*n* = 8), tumor growth was rapid. Median survival was 21 days in control groups (*n* = 55) and 54 days in the treatment (3F8 plus β-glucan) group (*n* = 82; *P* < 0.001; Fig. 6). Long-term survival was estimated at 18% in the treatment group and 0% in the controls.
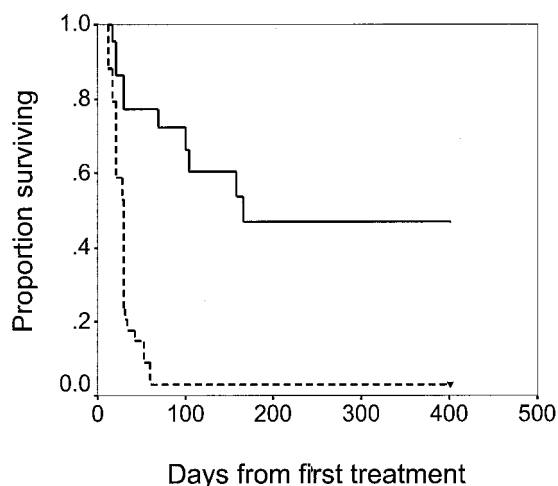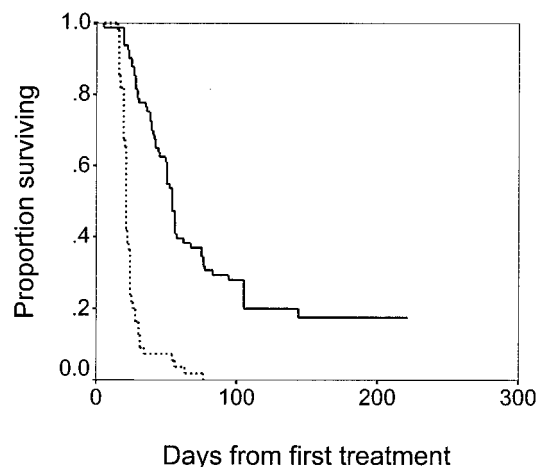
Fig. 5 Barley β-glucan synergized with 3F8 in prolonging survival from NMB7 neuroblastoma. Nude mice (*n* = 22) with established neuroblastoma NMB7 xenografts treated with 3F8 and barley β-glucan (———) had significantly longer median survival (median 166 days) compared with control mice (*n* = 34, ----, median 30 days) treated with saline alone (*n* = 10), 3F8 alone (*n* = 8), or β-glucan alone (*n* = 16; *P* < 0.001). Long-term survival was 47% in the treatment group and 3% in the control group.

Fig. 6 Barley β-glucan synergized with 3F8 in prolonging survival from LAN-1 neuroblastoma. In nude mice bearing established LAN-1 xenografts, median survival increased from 21 days in the control group (*n* = 55, ----) to 54 days in the 3F8 plus glucan group (*n* = 82, ———, *P* < 0.001).

DISCUSSION

Using the human xenograft models, we have made the following observations. β-Glucan derived from barley can synergize with monoclonal antibodies to suppress and/or eradicate tumors, whereas β-glucan or antibody alone has little antitumor effect. Antitumor response requires antibodies that activate complement, and both mouse IgM and mouse IgG3 were effective. p.o. administration of β-glucan is at least equally (if not more) effective than the i.p. route. It is a dose-dependent phenomenon, where ≥400 μg/dose is required for maximal effect. NK cells are not essential for this β-glucan phenomenon, although they contribute to the antitumor effect. Normal T cells and B cells are not required for the antitumor effect because immune-deficient mouse strains demonstrate the β-glucan effect, in both athymic and SCID-beige mice. Most importantly, p.o. β-glucan is well tolerated by all of the mice tested thus far, with no noticeable change in body weight, blood counts, or organ histologies, even at doses as high as 4 mg/dose/day.

Our findings differ significantly from previous observations and predictions on the use of β-glucans in cancer treatment. In the past, it was thought that the (1→3), (1→6)-β linkage was absolutely required for the β-glucan antitumor effect (13). This structure contains (1→3)-β-D-glucopyranosyl units, along which are randomly dispersed single β-D-glucopyranosyl units attached by (1→6)-linkages, giving a comb-like structure (*e.g.,* Lentinan, Schizophyllan, Laminarin, and glucan from Baker's yeast). In these models, it was believed that T cells were activated and indeed required for the antitumor effect. In addition, it was believed that small MW β-glucan should be more effective than high MW β-glucan and that the most effective administration should be i.v. or i.p. routes. Indeed, Betafectin was derived from a genetically engineered *S. cerevi-*

siae, which makes (1→3),(1→4)-β-D-glucans with weaker interchain associations (25). It was manufactured for i.v. injection to improve macrophage function in the hope of reducing infectious complications and improving wound healing. Barley β-glucan is a linear polymer with (1→3)-β and (1→4)-β linkages; however, it is not a comb-like structure. We did not find any antitumor effect of barley β-glucan when given alone. However, when used in combinations with monoclonal antibodies, the synergistic effect was remarkable. Although β-glucan activates granulocyte-mediated ADCC *in vitro* (data not shown), the effects of β-glucan may be indirect. It is not clear if the absorption of β-glucan is necessary for its antitumor effect. The exact mechanism of how β-glucan enhances the antitumor effect of monoclonal antibodies *in vivo* is unknown.

Monoclonal antibodies, either through Fc interaction or through CR3 interaction with iC3b, target cytotoxicity to tumor cells, a process greatly enhanced by β-glucan activation of effector cells. β-glucan activates leukocytes by binding to CR3 or β-glucan receptors (26). After antibodies deposit complement components on pathogens or cancer cells, C3b is proteolyzed rapidly into iC3b fragments by serum factor I. These iC3b fragments then opsonize the pathogens or tumor cells for the iC3b-receptors (CR3, CD11b/CD18) on phagocytic cells and NK cells, stimulating phagocytosis and/or cytotoxic degranulation. Thus, antibody and complement link innate and adaptive immunity by targeting antigens to different cells of the immune system, *e.g.,* via CR3 and Fc for phagocytic cells, CR2 for B cells, and CR1, CR2, or CR3 for follicular dendritic cells (27). For neutrophils, CR3-dependent phagocytosis requires ligation of two distinct binding sites, one for iC3b and a second site for β-glucan. Without β-glucan, iC3b-opsonized target cells are resistant to killing (17). Microbes possess polysaccharides that can activate the lectin domain on CR3, leading to phagocytosis and cytotoxic degranulation. In contrast, human cells (including tumors) lack these CR3-binding polysaccharides; thus, the inability of CR3 to mediate phagocytosis or extracellular cytotox-

icity of tumor cells opsonized with iC3b. The lectin site of CR3 can also influence transmembrane signaling of endogenous neutrophil membrane glycosylphosphatidyl inositol-anchored glycoproteins (*e.g.,* CD14, CD16, and CD59). In a mouse mammary tumor model, where there are naturally occurring IgM and IgG antibodies, injection of yeast soluble β-glucan could suppress tumor growth, an effect lost in C3-deficient or CD11b (CR3)-deficient mice (14, 28). Because iC3b bound to a primary protein antigen can also enhance recognition and specific antibody synthesis by antigen-specific B cells (29), the presence of β-glucan plus complement activation may enhance B-cell response to pathogens or tumor cells.

Another mechanism of action of β-glucan may relate to innate receptors for β-glucan in a hard-wired information network on phagocytes and lymphoid cells, receptors that normally recognize death signals and microbial molecular patterns (30). These innate receptors are biosensors for invading pathogens widely distributed in vertebrate and invertebrate animals (31), a nonclonal host defense pathway with structural and functional homologies in phylogenetic lineages that diverged over a billion years ago. After β-glucan activation of leukocytes, killing is immediate, nonclonal, and obligatory, a process often referred to as innate immunity. The consequence of this innate effector arm is the activation of costimulatory molecules and induction of cytokines and chemokines that will enhance adaptive immunity to the tumor cells (32, 33). Thus, antibodies, complement, phagocytes, and "danger" receptors form core elements of innate immunity, whereas antigen-presenting cells and T and B lymphocytes constitute essential players in acquired immunity.

Despite the availability of tumor-selective monoclonal antibodies and the ample supply of phagocytes/NKs, shrinkage of established tumors after antibody treatment alone and the acquisition of specific immunity are not common in both preclinical models and cancer patients. The absence of a danger signal and the diminution of complement action by complement resistance proteins on tumor cells may explain the inefficiency of antibody-mediated clinical responses (34). Lipopolysaccharide and β-glucan, being cell wall components of bacteria and fungus, respectively, are potent danger signals to the immune systems in all life-forms, from *Drosophila* to man (35). Although lipopolysaccharide is too toxic for human use, β-glucan is relatively benign. If this synergistic effect of β-glucan on antibodies is active in humans, our findings may have broad clinical implications: (*a*) the efficacy of monoclonal antibodies in cancer (*e.g.,* Herceptin, Rituximab, Dacluzimab, anti-GD2, and anti-EGF-R MoAb) can be potentially enhanced (36); nevertheless, although toxicity from β-glucan is expected to be minimal, the enhanced efficacy of MoAb may also increase MoAb-mediated toxicity, *e.g.,* the side effects of Herceptin on cardiac function, or anti-GD2 MoAb on neuropathic pain may be increased; and (*b*) because the amount and quality of barley and oat glucan in daily food intake can vary, future interpretations of efficacy trials using MoAb may need to take this into account, for both preclinical and clinical studies. Indeed, because glucan synergizes equally well with IgM antibody, the presence of natural IgM antitumor and antiviral antibodies can be a confounding factor in the interpretation of *in vivo* tumor response, whether in preclinical models or in clinical trials, unless the p.o. intake of glucan in mouse chow is standardized.

Most importantly, because many carbohydrate tumor vaccines [*e.g.,* GM2-KLH (37), GD2-KLH, and globo-H-hexasaccharide (38)] primarily induce specific IgM response, glucan may enhance their antitumor effects. In view of these potential beneficial effects of barley glucan on cancer therapy, a better understanding of their immune effects seems highly worthwhile.

REFERENCES

1. Schlom, J. Monoclonal antibodies in cancer therapy: basic principles. *In:* V. T. DeVita, S. Hellman, and S. A. Rosenberg (eds.), Biologic Therapy of Cancer, Ed. 2, pp. 507–520. Philadelphia: J. B. Lippincott Co., 1995.

2. Koehler, G., and Milstein, C. Continuous culture of fused cells secreting antibody of predefined specificity. Nature (Lond.), *256:* 495–496, 1975.

3. Riethmuller, G., Holz, E., Schlimok, G., Schmiegel, W., Raab, R., Hoffken, K., Gruber, R., Funke, I., Pichlamaier, H., Hirche, H., Buggisch, P., Witte, J., and Pichlmayr, R. Monoclonal antibody therapy for resected Dukes' C colorectal cancer: seven-year outcome of a multicenter randomized trial. J. Clin. Oncol., *16:* 1788–1794, 1998.

4. Maloney, D. G., Grillo-Lopez, A. J., Bodkin, D. J., White, C. A., Liles, T-M., Royston, I., Varns, C., Rosenberg, J., and Levy, R. IDEC-C2B8: results of a Phase I multiple-dose trial in patients with relapsed non-Hodgkin's lymphoma. J. Clin. Oncol., *15:* 3266–3274, 1997.

5. McLaughlin, P., Grillo-Lopez, A. J., Kink, B. K., Levy, R., Czuczman, M. S., Williams, M. E., Heyman, M. R., Bence-Bruckler, I., White, C. A., Cabanillas, F., Jain, V., Ho, A. D., Lister, J., Wey, K., Shen, D., and Dallaire, B. K. Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to four-dose treatment program. J. Clin. Oncol., *16:* 2825–2833, 1998.

6. Cobleigh, M. A., Vogel, C. L., Tripathy, D., Robert, N. J., Scholl, S., Fehrenbacher, L., Wolter, J. M., Paton, V., Shak, S., Lieberman, G., and Slamon, D. J. Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. J. Clin. Oncol., *17:* 2639–2648, 1999.

7. Slamon, D. J., Leylan-Jones, B., Shak, S., Fuchs, H., Paton, V., Bajamonde, A., Fleming, T., Eiermann, W., Wolter, J., Pegram, M., Baselga, J., and Norton, L. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpress HER2. N. Engl. J. Med., *344:* 783–792, 2001.

8. Jurcic, J. G., DeBflasio, T., Dumont, L., Yah, T. J., and Scheinberg, D. A. Molecular remission induction with retinoic acid and anti-CD33 monoclonal antibody HuM195 in acute promyelocytic leukemia. Clin. Cancer Res., *6:* 372–380, 2000.

9. Yeh, S. D., Larson, S. M., Burch, L., Kushner, B. H., LaQuaglia, M., Finn, R., and Cheung, N. K. V. Radioimmunodetection of neuroblastoma with iodine-131–3F8: correlation with biopsy, iodine-131-Metaiodobenzylguanidine (MIBG) and standard diagnostic modalities. J. Nucl. Med., *32:* 769–776, 1991.

10. Cheung, N. K. V., Kushner, B. H., Cheung, I. Y., Canete, A., Gerald, W., Liu, C., Finn, R., Yeh, S. J., and Larson, S. M. Anti-GD2 antibody treatment of minimal residual stage 4 neuroblastoma diagnosed at more than 1 year of age. J. Clin. Oncol., *16:* 3053–3060, 1998.

11. Lanzavecchia, A., Abrignani, S., Scheidegger, D., Obrist, R., Dorken, B., and Moldenhauer, G. Antibodies as antigens. The use of mouse monoclonal antibodies to focus human T cells against selected targets. J. Exp. Med., *167:* 345–352, 1988.

12. Jerne, N. K. Toward a network theory of the immune system. Ann. Immunol. (Paris), *125C:* 373–389, 1974.

13. Bohn, J. A., and BeMiller, J. N. (1–3)-β-D-glucans as biological response modifiers: a review of structure-functional activity relationships. Carbohydr. Polym., *28:* 3–14, 1995.

14. Xia, Y., Vetvicka, V., Yan, J., Hanikyrova, M., Mayadas, T., and Ross, G. D. The β-glucan-binding lectin site of mouse CR3 (CD11b/CD18), and its function in generating a primed state of the receptor that mediates cytotoxic activation in response to iC3b-opsonized target cells. J. Immunol., *162:* 2281–2290, 1999.

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

16. Vetvicka, V., Thornton, B. P., Wieman, T. J., and Ross, G. D. Targeting of natural killer cells to mammary carcinoma via naturally occurring tumor cell-bound iC3b and β-glucan-primed CR3 (CD11b/CD18). J. Immunol., *159:* 599–605, 1997.

17. Vetvicka, V., Thornton, B. P., and Ross, G. D. Soluble β-glucan polysaccharide binding to the lectin site of neutrophil or natural killer cell complement receptor type 3 (CD11b/CD18) generates a primed state of the receptor capable of mediating cytotoxicity of iC3b-opsonized target cells. J. Clin. Investig., *98:* 50–61, 1996.

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

19. Thornton, B. P., Vetvicka, V., Pitman, M., Goldman, R. C., and Ross, G. D. Analysis of the sugar specificity and molecular location of the β-glucan-binding lectin site of complement receptor type 3 (CD11b/CD18). J. Immunol., *156:* 1235–1246, 1996.

20. Roubroeks, J. P., Skjak-Braek, G., Ryan, L., and Christensen, B. E. Molecular weight dependency on the production of the TNF stimulated by fractions of rye (1→3), (1→4)-β-D-glucan. Scand. J. Immunol., *52:* 584–587, 2000.

21. Cheung, N. K., Saarinen, U., Neely, J., Landmeier, B., Donovan, D., and Coccia, P. Monoclonal antibodies to a glycolipid antigen on human neuroblastoma cells. Cancer Res., *45:* 2642–2649, 1985.

22. Modak, S., Kramer, K., Humayun, G., Guo, H. F., and Cheung, N. K. V. Monoclonal antibody 8H9 targets a novel cell surface antigen expressed by a wide spectrum of human solid tumors. Cancer Res., *61:* 4048–4054, 2001.

23. Nethery, A., Raison, R. L., and Easterbrook-Smith, S. B. Single-step purification of immunoglobulin M on C1q-sepharose. J. Immunol. Methods, *126:* 57–60, 1990.

24. Cheung, N. K. V., Walter, E. I., Smith-Mensah, W. H., Ratnoff, W. D., Tykocinski, M. L., and Medof, M. E. Decay-accelerating factor protects human tumor cells from complement-mediated cytotoxicity *in vitro*. J. Clin. Investig., *81:* 1122–1128, 1988.

25. Zimmerman, J. W., Lindermuth, J., Fish, P. A., Palace, G. P., Stevenson, T. T., and DeMong, D. E. A novel carbohydrate-glycosphingolipid interaction between a β-(1-3)-glucan immunomodulator, PGG-glucan, and lactosylceramide of human leukocytes. J. Biol. Chem., *273:* 22014–22020, 1998.

26. Mueller, A., Raptis, J., Rice, P. J., Kalbfleisch, J. H., Stout, R. D., Ensley, H. E., Browder, W., and Williams, D. L. The influence of glucan polymer structure and solution conformation on binding to (1→3)-β-D-glucan receptors in a human monocyte-like cell line. Glycobiology, *10:* 339–346, 2000.

27. Vivanco, F., Munoz, E., Vidarte, L., and Pastor, C. The covalent interaction of C3 with IgG immune complexes. Mol. Immunol., *36:* 843–852, 1999.

28. Yan, J., Vetvicka, V., Xia, Y., Coxon, A., Carroll, M. C., Mayadas, T. N., and Ross, G. D. β-Glucan a "specific" biologic response modifier that uses antibodies to target tumors for cytotoxic recognition by leukocyte complement receptor type 3 (CD11b/CD18). J. Immunol., *163:* 3045–3052, 1999.

29. Thornton, B. P., Vetvicka, V., and Ross, G. D. Function of C3 in a humoral response: iC3b/C3dg bound to an immune complex generated with natural antibody and a primary antigen promotes antigen uptake and the expression of costimulatory molecules by all B cells, but only stimulates immunoglobulin synthesis by antigen-specific B cells. Clin. Exp. Immunol., *104:* 531–537, 1996.

30. Matzinger, P. Tolerance, danger, and the extended family. Annu. Rev. Immunol., *12:* 991–1045, 1994.

31. Medzhitov, R., and Janeway, C. A. An ancient system of host defense. Curr. Opin. Immunol., *10:* 12–15, 1998.

32. Fuchs, E. J., and Matzinger, P. Is cancer dangerous to the immune system? Semin. Immunol., *8:* 271–280, 1996.

33. Matzinger, P. An innate sense of danger. Semin. Immunol., *10:* 399–415, 1998.

34. Liszewski, M. K., Farries, T. C., Lublin, D. M., Rooney, I. A., and Atkinson, J. P. Control of the complement system. Adv. Immunol., *61:* 201–283, 1996.

35. Kim, Y. S., Ryu, J., Han, S., Choi, K., Nam, K., Jang, I., Lemaitre, B., Brey, P. T., and Lee, W. Gram-negative bacteria binding protein, a pattern recognition receptor for lipopolysaccharide and β-1,3-glucan, which mediates the signaling for the induction of innate immune genes in *Drosophila melanogaster* cells. J. Biol. Chem., *275:* 32721–32727, 2000.

36. Jurianz, K., Maslak, S., Garcia-Schuler, H., Fishelson, Z., and Kirschfink, M. Neutralization of complement regulatory proteins augments lysis of breast carcinoma cells targeted with rhumAb anti-HER2. Immunopharmacology, *42:* 209–218, 1999.

37. Livingston, P. O., Wong, G. Y. C., Adluri, S., Tao, Y., Padavan, M., Parente, R., Hanlon, C., Calves, M. J., Helling, F., Ritter, G., Oettgen, H. F., and Old, L. J. Improved survival in stage III melanoma patients with GM2 antibodies: a randomized trial of adjuvant vaccination with GM2 ganglioside. J. Clin. Oncol., *12:* 1036–1044, 1994.

38. Slovin, S. F., Ragupathi, G., Adluri, S., Ungers, G., Terry, K., Kim, S., Spassova, M., Bornmann, W. G., Fazzari, M., Dantis, L., *et al.* Carbohydrate vaccines in cancer: immunogenicity of a fully synthetic globo H hexasaccharide conjugate in man. Proc. Natl. Acad. Sci. USA, *96:* 5710–5715, 1999.