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Antitumor Effect of Soluble β-1,3-Glucan from *Agrobacterium* sp. R259 KCTC 1019

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Abstract β-1,3-Glucans enhance immune reactions such as antitumor, antibacterial, antiviral, anticoagulatory, and wound healing activities. β-1,3-Glucans have various functions depending on the molecular weight, degree of branching, conformation, water solubility, and intermolecular association. The molecular weight of the soluble glucan was about 15,000 as determined by a high-performance size exclusion chromatography. From the infrared (IR) and ^{13}C NMR analytical data, the purified soluble glucan was found to exclusively consist of β-D-glucopyranose with 1,3 linkage. We tested the immune-stimulating activities of the soluble β-1,3-glucan extracted from *Agrobacterium* sp. R259 KCTC 1019 and confirmed the following activities. IFN-γ and each cytokines were induced in the spleens and thymus of mice treated with soluble β-1,3-glucan. Adjuvant effect was observed on antibody production. Nitric oxide was synthesized in monocytic cell lines treated with β-1,3-glucan. The cytotoxic and antitumor effects were observed on various cancer cell lines and ICR mice. These results strongly suggested that this soluble β-1,3-glucan could be a good candidate for an immune-modulating agent.

Keywords: β-1,3-Glucans, cytokine, adjuvant, antitumor, immune-stimulating activity

β-1,3-Glucans exhibit enhancing effects on various immunological activities [6, 8]. Some of the glucans, such as lentinan and sonifilan, have been clinically used for cancer therapy in Japan. Most experimental evidences suggest that many β-1,3-glucans have immunopharmacological activities in which the significance is dependent on molecular weight, degree of branching, and conformation [30]. The conformation of β-1,3-glucans has been assumed to be one of the contributing factors to the biological activity of the glucan [11]. Since most β-1,3-glucans are usually insoluble in water, their biological activities have been studied as particulate materials, such as zymosan and zymocel. It was also known that biological activities of soluble β-1,3-glucans are significantly different from those of particulate β-1,3-glucans [1]. The mitogenic activity of β-1,3-glucans results in several immune responses. These responses include increased natural killer (NK) cell activity, T cell-mediated cytotoxicity, proliferative responses of peripheral blood mononuclear cells (PBMCs) to mitogen, a stimulated release of cytokines such as interferons (IFNs) and interleukins (IL), and induction of phagocytotic activity of neutrophils [23]. β-1,3-Glucan was reported to induce nonspecific protection against tumors and microorganisms [25]. We have detected immune-stimulating activity of the soluble 1,3-glucan extracted from *Agrobacterium* sp. R259 KCTC 10197BP (Korean Collection for Type Cultures, The Genetic Resources Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon, Korea).

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Materials and Methods

Mice and *Agrobacterium* sp. R259 KCTC 10197BP

The Genetic Resources Center, Korea Research Institute of Bioscience and Biotechnology, provided four-week-old male Balb/c and ICR mice. The mice were kept under specific pathogen-free condition. *Agrobacterium* sp. R259 KCTC 10197BP was also provided from the Genetic Resources Center.

Preparation of β-Glucan by Enzymatic Hydrolysis

The enzymatically hydrolyzed form of β-glucan from *Agrobacterium* sp. R259 KCTC 1019BP (DMJ-M) was used and labelled as DMJ-E in this study. Culture conditions

and the method of DMJ-M preparation have been described elsewhere [12]. *Cellulomonas* sp. ATCC 21712, which produced glucanase, was cultivated in YCWD medium (10 g tryptone, 1 g yeast extract per 1 l of distilled water) including 1% DMJ-M at 32°C for 48 h. The sample was centrifuged at 8,000 ×*g* for 15 min. DMJ-M was added to the supernatant at a concentration of 2% and the mixture was incubated at 40°C for 48 h in a shake flask. It was centrifuged at 8,000 ×*g* for 15 min and the insoluble fraction was discarded. Glucanases were deactivated by heat treatment of the supernatant at 70°C for 20 min. The sample was treated with three volumes of ethanol at 4°C for 8 h. It was centrifuged at 30,000 ×*g* for 30 min. The precipitated glucan was harvested by centrifuging at 5,000 ×*g* for 15 min, and washed three times with ethanol to remove salts. To obtain the white powder of glucan, it was freeze-dried and ground to fine powder. For investigating structural features of glucan in all experiments, the purified glucan (DMJ-E) was dissolved in distilled-deionized water.

Analyses of Molecular Structure

The molecular weight was determined by size exclusion high-performance liquid chromatography (HPLC). The chromatographic system consisted of an HPLC pump (Waters 501; Milford, MA, U.S.A.), a differential refractometer (Waters 410), and a data module (Waters 746). The chromatograms were obtained with two series of PL-GFC columns (300×7.5 mm i.d., 8-μm particle size, 300 Å pore size; Polymer Laboratories Inc., Amherst, MA, U.S.A.). The mobile phase of water was pumped at a flow rate of 1.0 ml/min. The system was operated at 50°C. Dextrans (MW; 505, 1,200, 5,700, 37,500, 60,000–90,000, and 143,000), purchased from Sigma, were used as standard samples. The composition of DMJ-E was determined by the analysis of the monosaccharide using gas chromatography-mass spectrometry (GC-MS). DMJ-E was hydrolyzed with 2.5 M trifluoroacetic acid, which derivatized the monosaccharides into their alditol acetate forms. Alditol acetates were recovered with CH_2Cl_2 and analyzed by GC-MS. The GC-MS system analyses were performed on a Hewlett-Packard 5890 Series II gas chromatograph (Hewlett-Packard, Avondale, U.S.A.) with a JMS-AX 505 WA mass spectrometer using a DB-23 capillary column (30 m×0.215 mm i.d.×0.25 μm; J&W Scientific Inc., CA, U.S.A.) with He carrier gas. The column temperature was 220°C at an isocratic mode. Myoinositol was used as the internal standard. Infrared spectra were obtained on a Matton Fourier Transform-Infrared (FT-IR) spectrophotometer (Thermomattson, Madison, WI, U.S.A.) employing potassium bromide (KBr) discs. ^{13}C NMR data were obtained on a JEOL JNM-LA 400 spectrometer (Jeol Co., Ltd., Tokyo, Japan) operating at 100 MHz. The sample of DMJ-E was dissolved in distilled-deionized water.

RT-PCR

Balb/c mice were i.p. injected daily with DMJ-E 250 μg for three days. Spleens and thymi were removed from half of the mice a day after the 3rd injection. The rest of the mice were injected with 10 μg LPS and then spleens and thymi were removed after 12 h. Total cellular RNA was extracted from the spleens and thymi by an acid guanidinium thiocyanate-phenol-chloroform extraction method. Seven μg of RNA was mixed with 4 μl of $p(dT)_{15}$ primer (100 ng/μl, Roche), 1.5 μl of reverse transcriptase (50 U/μl, Stratagene), 6.5 μl of 10× reaction buffer, and 3 μl of 100 mM dNTP (Roche) in a final volume 65μl and incubated at 37°C for 1.5 h and 95°C for 5 min. The primers used for this study were an IL-6 sense primer (S) 5-CTC TGC AAG AGA CTT CCA TC-3 and antisense primer (AS) 5-GCC GAG TAG ATC TCA AAG TG-3, IL-1Ra (S) 5-CTT CTG TTT CAT TCA GAG GC-3, and (AS) 5-GAT GCC CAA GAA CAC ACT AT-3. For GAPDH, IL-1β, and IL-2, primers were used as previously described [5]. PCR was performed in a final volume of 50 μl containing a cDNA template, 10 pmol sense primer and antisense primer, 0.5 mM dNTP, and Taq polymerase. PCR was performed in a GeneAmp PCR System 9600 (Perkin Elmer, MA, U.S.A.). The GAPDH, IL-1Ra, TNF-α, IL-1β, IL-6, and IL-2 genes were denatured at 94°C for 10 min, and then 30 cycles of amplification (94°C for 1 min, 60°C for min, and 72°C for 15 min) were performed followed by a 10 min extension at 72°C.

IFN-γ Assay of PBMCs

Human PBMCs in RPMI 1640 medium were seeded into 96-well plates at 100 μl, at final concentrations of 1×10^6/ml, and pretreated with polymyxin B (10 μg/ml) to neutralize endotoxins. LPS (5 μg/ml), IL-18 (50 ng/ml), and PHA (1 mg/ml) were used as costimulators. PBMCs treated with DMJ-E were incubated at 37°C in a humidified incubator with 5% CO_2 for 24 h. The amount of the IFN-γ was measured with an OptEIA human IFN-γ ELISA kit (Pharmingen, U.S.A.) according to the manufacturer's instructions.

Cell Culture

A human monocyte cell line (THP-1) was maintained in RPMI-1640 medium (GibcoBRL, NY, U.S.A.) containing 10% heat-inactivated fetal bovine serum (FBS). The cells were stimulated with and without DMJ-E (50, 100, or 20 μg/ml) prior to addition of zymosan (50 μg/ml) at a cell density of 4×10^4 cells/well. The HPV-18-positive cervical cancer cell line HeLa, HPV-16-positive cervical cancer cell lines such as CaSki and C3, hepatoma cancer cell line HepG2, and Sarcoma-180 were purchased from American Type Culture Collection (Rockville, MD, U.S.A.). These cell lines were maintained in Dulbecco's Modified Eagle's

Medium (GibcoBRL). The cells were stimulated with and without DMJ-E (40, 80, and 120 μg/ml).

Administration of Antigen and DMJ-E

In order to investigate the adjuvant effect of DMJ-E, E6 and E7 recombinant proteins [4] as antigens and DMJ-E (250 μg) were i.p. injected into mice once a week, on days 0, 7, 14, and 21. The control mice were injected with pyrogen-free saline alone under the same conditions as β-glucan-treated mice. Sera were prepared on day 26.

Preparation of Mouse Sera

Mouse blood was obtained by cardiopuncture and incubated at room temperature for 1 h. After the blood was centrifuged at 15,000 rpm for 10 min, the supernatant was used as a serum sample for E6 and E7 antibody titration.

Measurement of Nitric Oxide

After incubation for 24 h, synthesis and release of NO by monocyte cell line were both determined by an assay of the culture supernatant for nitrite content. One-hundred μl of supernatant was incubated with an equal volume of Griess reagent (1% sulfanilamide, 0.1% naphthylethylene-diamine dihydrochloride, 2.5% phosphoric acid) at room temperature for 10 min. The optical density was measured at 550 nm (reference 630 nm) [8]. The nitrite content was quantified by comparison with a standard curve generated with sodium nitrite in the range of 0–100 mM [14].

Cell Viability

Cells were assessed for their proliferating response to DMJ-E using detection of mitochondrial dehydrogenases, by cleaving of a novel tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxy methoxyphenyl)-2H tetrazolium, inner salt; MTS]. On the day of the proliferation assay, MTS/phenazine methosulfate (PMS) solution was prepared by mixing 25 μl PMS (1.53 mg/ml in PBS) for every 975 μl MTS (1.71 mg/ml in PBS). Fifty μl of PMS/MTS solution was added to the 96-well culture plate at 37°C for 1 to 3 h. The absorbance of formazan at 490 nm was measured directly from the 96-well assay plates without additional processing.

Antitumor Activity

Sarcoma-180 cells (5×10^6) were i.p. injected into ICR mice. DMJ-E was administered intraperitoneally every other day (7 times) at a dose of 250 μg. Three weeks after tumor inoculation, the number of surviving mice in each group was recorded.

Statistical Evaluation

Cellular viability data and an ELISA data were analyzed by Student's *t* test using Prism version 3.00 (GraphPad Software, San Diego, CA, U.S.A.).

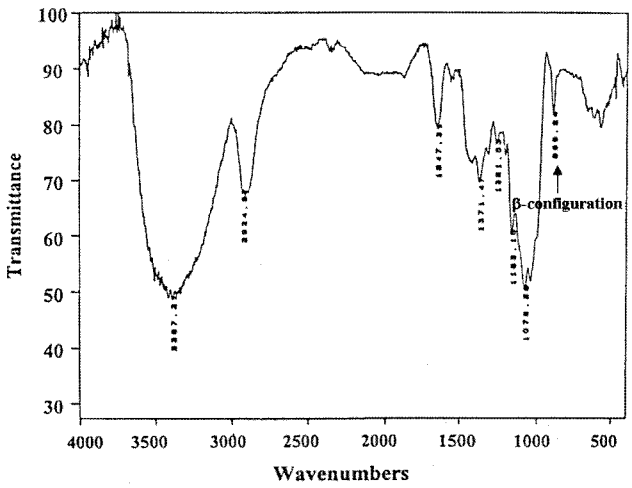

Fig. 1. FT-IR spectrum of DMJ-E.
Infrared spectra were obtained on a Matton Fourier Transform-Infrared (FT-IR) spectrophotometer (Thermomattson, Madison, WI, U.S.A.) employing potassium bromide (KBr) discs.

RESULTS

Structure Analysis

The molecular weight of DMJ-E was about 15,000, as determined by size exclusion high-performance liquid chromatography. The purified soluble glucan was found to consist exclusively of glucose from the analysis of the monosaccharide. To find the configuration of glucose, infrared (IR) spectroscopy was carried out. As shown in Fig. 1, the IR spectrum shows an absorption band at 890 cm^{-1}, indicating that D-glucopyranose has a β-configuration. It was concluded that no α-configuration exists since there was no characteristic absorption band at 840 cm^{-1}. Fig. 2 shows the ^{13}C NMR spectrum of the bacterial β-glucan. Correlations with carbon chemical shifts at 103.1, 72.8, 86.2, 68.4, 76.3, and 60.9 ppm are assigned as C-1, C-2, C-3, C-4, C-5, and C-6, respectively, which represent the (1→3)-β-D-glucan backbone in the polymer chain. From the NMR spectrum, it can also be

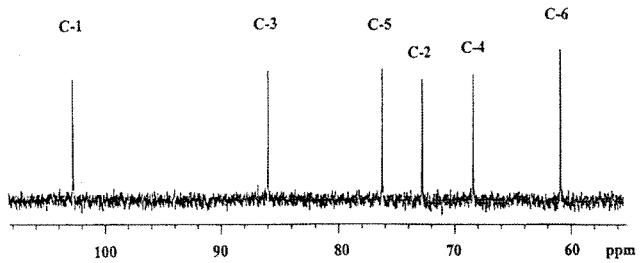

Fig. 2. ^{13}C NMR spectrum of DMJ-E. ^{13}C NMR data were obtained on a JEOL JNM-LA 400 spectrometer (Jeol Co., Ltd., Tokyo, Japan) operating at 100 MHz.
The sample of DMJ-E was dissolved in distilled-deionized water.

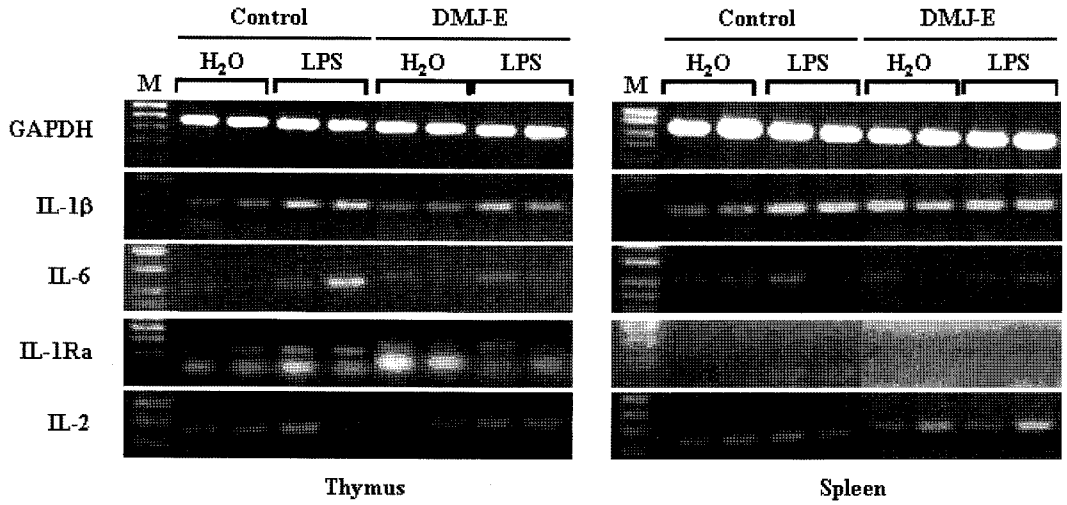

Fig. 3. Expression of cytokine mRNAs in the thymi and the spleens of Balb/c mice treated with DMJ-E in the presence or absence of LPS.

Balb/c mice were injected 3 times with DMJ-E (250 μg/mouse) and treated with or without LPS (10 μg/mouse). Total RNA extracted from thymi and spleens were subjected to RT-PCR and PCR products were electrophoresed on 1.5% RNA agarose gel.

concluded that the β-glucan has linear (1→3)-linkages, since evidence of other linkages was not seen in the spectrum.

Gene Expression of Cytokine mRNA in Balb/c Mice Treated with DMJ-E

The gene expression of cytokine mRNA was compared by RT-PCR using appropriate primers, as shown in the Materials and Methods. LPS-treated thymi showed that the expressions of mRNAs for IL-1, IL-6, and IL-1Ra were induced (Fig. 3). The IL-2 mRNA was slightly induced by LPS in thymi. TNF-α could not be detected in thymi and spleens (data not shown). β-1,3-Glucan DMJ-E enhanced the level of IL-1 mRNA in the spleen and reduced the level of IL-1Ra mRNA induced by LPS in the thymi. These results suggest that IL-1 mRNA was regulated by DMJ-E and, thus, DMJ-E can regulate IL-1-induced immune reactions.

Effect of DMJ-E on IFN-γ Production in PBMCs

Human PBMCs (1×10^6/ml) were pretreated with polymyxin B (10 μg/ml) to neutralize endotoxins. LPS (5 μg/ml), IL-18 (50 ng/ml), and PHA (1 μg/ml) were used as a costimulating agent. The amount of the IFN-γ was measured with an OptEIA human IFN-γ ELISA kit. The stimulating effect of DMJ-E on INF-γ production was investigated in isolated PBMCs (Fig. 4). LPS or IL-18 alone did not induce IFN-γ production. DMJ-E induced IFN-γ, whereas DMJ-E did not show significant comitogenic effect when treated with LPS or IL-18. There were no differences in IFN-γ production between LPS/DMJ-E (or IL-18/DMJ-E) cotreatment and DMJ-E treatment. The added LPS and LPS-costimulation was supposed to be inactive because the polymyxin B was added to all experiments using human PBMCs. These results support that there were no significant effects of endotoxin, which would be contaminated in during the process of purification of DMJ-E. In the case of the PHA/DMJ-E, IFN-γ production was remarkably stimulated to about 3 to 4 times that of PHA alone.

Effect of β-Glucan on NO Generation in Monocytic Cell Line

The NO produced by a THP-1 monocytic cell line was measured in the culture supernatant. The supernatant was

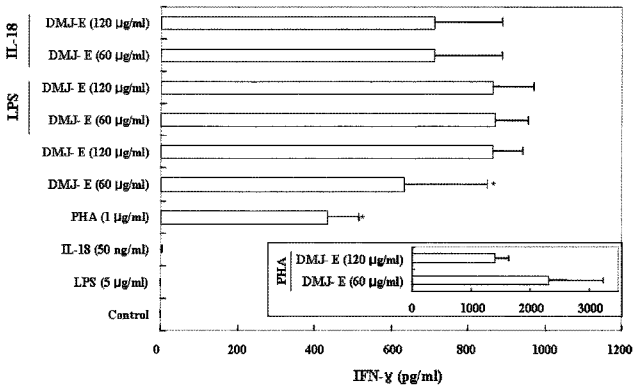

Fig. 4. The effect of DMJ-E on IFN-γ production in PBMCs in the presence or absence of costimulator.

Polymyxin B (10 μg/ml) was added to the cells to neutralize endotoxin. The concentrations of the reagents used were as follows: LPS (5 μg/ml), IL-18 (50 ng/ml), PHA (1 μg/ml), and DMJ-E (30, 60 μg/ml). PBMCs (1×10^7/well) were given PHA and DMJ-E, and incubated at 37°C for 24 h in a humidified 5% CO_2 incubator. Subsequently, the culture supernatants were collected and IFN-γ concentrations were measured as described in Materials and Methods. Measurements were done in triplicate, and the error bars represent SD. *, Significant difference from nontreated control cells ($p<0.05$).

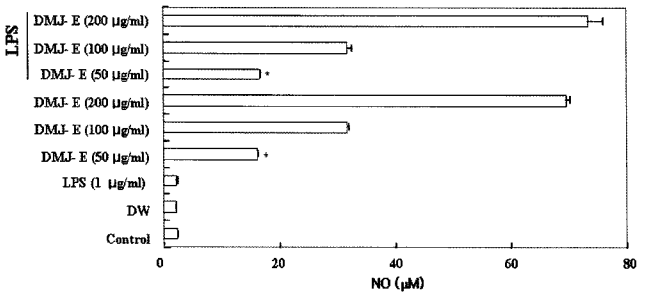

Fig. 5. The effects of DMJ-E on the NO production in THP-1 cells.
THP-1 cells (2×10^5/well) were incubated for 4 h with various doses of DMJ-E (50, 100, 200 μg/ml) in the presence or absence of LPS (1 μg/ml). After an additional 24 h incubation at 37°C in a humidified 5% CO_2 incubator, the culture supernatants were collected and nitric oxide concentrations were measured as described in Materials and Methods. Measurements were done in triplicate, and the error bars represent SD. *, Significant difference from nontreated control cells ($p<0.05$).

incubated with an equal volume of Griess reagent at room temperature for 10 min. The optical density was measured at 550 nm (reference 630 nm). DMJ-E induced NO production in THP-1 in a dose-dependent manner. DMJ-E-treated THP-1 produced NO as much as DMJ-E- and LPS-treated THP-1 cells did (Fig. 5).

Effect of DMJ-E on the Antibody Production Against E6 and E7 in Mice

To examine the activity of DMJ-E on the antibody production against exogenous antigen, mice were administered with E6 or E7 and DMJ-E on days 0, 7, 14, and 21. Blood was obtained by cardiopuncture on day 26. The antibody levels in sera were measured by ELISA using E6- or E7-coated plates. As shown in Table 1 or Fig. 6, treatment with either only E6 or E7 illustrated titers of about 1,600 and 2,400 respectively, whereas both E6/E7 together with β-glucan exhibited higher titers of about 3,250 and 8,533, respectively. E6 or E7 with the MPL+TDM Adjuvant system (Sigma, Saint Louis, MS, U.S.A.) exhibited titers of about 140,800 and 22,400, respectively. These results indicated that DMJ-E plays an adjuvant role in antibody production.

Table 1. Comparison of DMJ-E as an adjuvant for Ab production against E6 and E7 antigens.

	E6 Ab Titer	E7 Ab Titer
Normal	-	-
DW	1,600	2,400
DMJ-E	3,250	8,533
MPL+TDM	140,800	22,400

E6 (20 μg/mouse) or E7 (20 μg/mouse) and DMJ-E (250 μg/mouse), MPL+TDM (100 μl/mouse), or saline were i.p. administered to ICR mice on days 0, 7, 14, and 21. Blood was obtained by cardiopuncture on day 26. The antibody levels in sera were measured by ELISA.

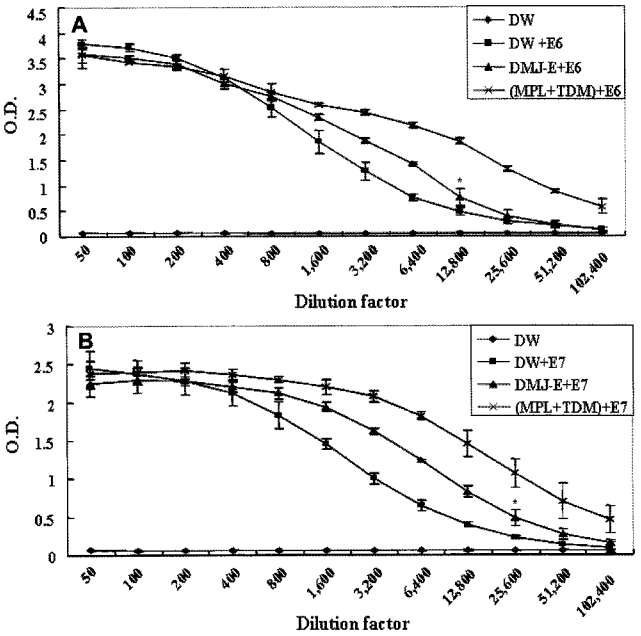

Fig. 6. The adjuvant effects of DMJ-E on antibody titers against E6 and E7 antigens.
E6 (20 μg/mouse) (**A**) or E7 (20 μg/mouse) (**B**) and DMJ-E (250 μg/mouse), MPL+TDM (100 μl/mouse), or saline were i.p. administerd to ICR mice on days 0, 7, 14, and 21. Blood was obtained by cardiopuncture on day 26. The antibody levels in sera were measured by ELISA. Measurements were done in triplicate, and the error bars represent SD. *, Significant difference from E6 or E7 (emulsified in DW)-treated controls ($p<0.05$).

Cytotoxic Effect of DMJ-E on the Tumor Cells

The cytotoxic effect of DMJ-E was assessed *in vitro*. Various cancer cell lines, originating from the cervix, liver, and ascites, were tested as described in Materials and Methods. Among the cervical cancer cell lines, HeLa, CaSki, and C3, the viability of CaSki and C3 was reduced by 20%–30%. There was almost no effect on hepatitis cancer cell line HepG2, whereas the viability of ascite tumor cell line Sarcoma-180 was decreased by 30%–40% (Fig. 7).

Antitumor Effect of DMJ-E on the Ascites-Form Tumor, Sarcoma-180

Sarcoma-180 cells (5×10^6) were inoculated into the intraperitoneal cavity of ICR mice. DMJ-E (250 μg) was

Table 2. Antitumor activity of DMJ-E against Sarcoma-180 ascites tumor in ICR mice.

	No. of survivors/total			
	<3 weeks	<4 weeks	<7 weeks	<9 weeks
DW	0/7			
DMJ-E	3/7	2/7	2/7	2/7

Sarcoma-180 tumor cells (5×10^6/mouse) were inoculated into ICR mice on day 0. DMJ-E (250 μg/mouse) or distilled water (DW) were i.p. administered to the same mice 7 times. For nine weeks after tumor inoculation, survival rate was checked and the values in the table represent the number of survivors.

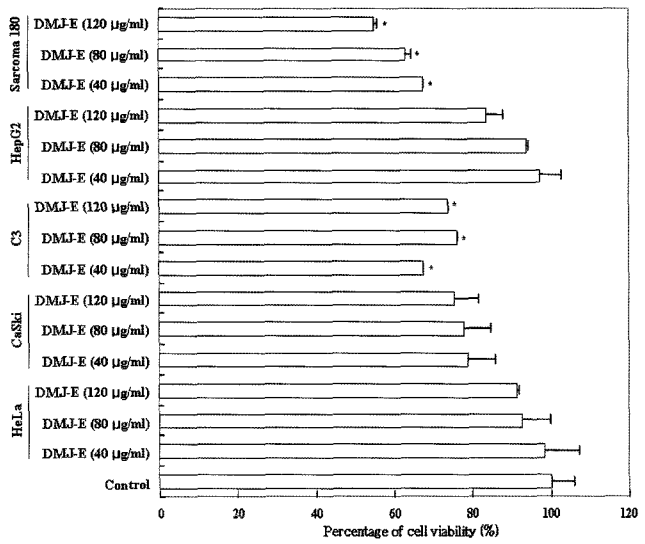

Fig. 7. The effect of DMJ-E on the viability of various cells.
HeLa, CaSki, C3, HepG2, and Sarcoma-180 cells (1×10^5/well) were preincubated at 37°C for 24 h in humidified 5% CO_2. Different doses of DMJ-E (40, 80, 120 μg/ml) were administered to cells for 48 h, respectively. Then the viability of cells was detected by using MTS/PMS reagent as described in Materials and Methods. Measurements were done in triplicate, and the error bars represent SD. *, Significant difference from nontreated control cells ($p<0.05$).

administered intraperitoneally every other day (7 times). As shown in Table 2, life span clearly increased in DMJ-E-treated groups, compared with nontreated groups.

DISCUSSION

To support the basic theory for widespread use, a variety of experiments including cytokine production [13, 16], adjuvant activity [15], NO generation, and antitumor activity [19, 27, 31, 32] have been investigated. The immunological and antitumor activity of certain β-(1,3)-glucan biological response modifiers (BRMs) are related to the polymer structure. DMJ-E is an enzymatically hydrolyzed form of β-glucan from *Agrobacterium* sp. R259 KCTC 1019BP. These studies show that the main structural components of the DMJ-E are entirely composed of D-glucosyl residues, which are connected almost exclusively by β-(1,3)-linkages. Thus, in order to expand the immunopharmacological property of DMJ-E, we have screened several activities in this study, and found that the β-glucan DMJ-E has an ability to enhance various immune-stimulating activities. Considering that cytokines play important roles in immune and inflammatory reaction, we have investigated a wide variety of cytokines from spleen, thymi, and PBMCs. DMJ-E induced IFN-γ production in PBMCs. IFN-γ, a cytokine derived primarily from activated T lymphocytes and natural killer (NK) cells, plays a central role in the modulation of both innate and acquired immune responses. IFN-γ is regarded as a potent activator of macrophage-mediated antimicrobial functions, such as phagocytosis, respiratory burst activity, antigen presentation, and production of pro-inflammatory cytokines [16, 22]. However, the mechanism for IFN-γ induction by the glucan has not yet been reported [10]. β-1,3-Glucan stimulated the induction of each cytokines such as IL-1β, IL-6, IL-1Ra, and IL-2 from the thymus and spleen. The mRNA levels of induced cytokines were similar to those obtained with LPS stimulated tissues (Fig. 3). As shown in Fig. 3, β-1,3-glucan DMJ-E enhanced the level of IL-1 mRNA in the spleen and reduced the mRNA level of IL-1Ra induced by LPS in the thymi. These results suggest that β-1,3-glucan is a potent inducer of IL-1. IL-1 has multiple effects on cells involved in inflammation. Moreover, IL-1 can stimulate macrophages to exhibit a more prolonged and active tumorcidial state [21]. In addition, IL-1 itself may be directly cytocidal for certain tumor target cells [20]. Thus, it is assumed that IL-1 secreted by β-glucans-activated monocytes plays an important part in bacterial resistance and tumor resistance. IL-2, produced mainly by T-helper cells, also has many important immunologic functions [26]. IL-6 has a wide array of biological actions on lymphoid and nonlymphoid cells, which is important in host defense and inflammatory responses. IL-6-deficient mice are highly susceptible to infection by *Candida* and *Listeria* [28]. IL-6 induces the synthesis of acute-phase response proteins in hepatocytes, terminal differentiation of B cells to antibody-producing plasma cells, differentiation of monocytes to macrophages, and growth of hematopoietic stem cells [18].

Various substances are used as adjuvants in experimental models. The adjuvant is also important for controlling the Th1/Th2 balance. Some β-glucans showed an adjuvant effect on antibody production [13]. Our data showed an adjuvant effect on antibody generation against viral oncogenes E6 and E7. β-Glucans have the ability to induce NO synthesis by macrophages, and the intensity of NO synthesis significantly varied depending on the structure of β-glucans. DMJ-E induced NO generation in THP-1 cells in a dose-dependent manner.

In addition, we examined the inhibitory effect on various cancer cell lines and extensively on tumors. DMJ-E inhibited C3 cell proliferation by 30% at 40 μg/ml concentration. Similar results were obtained for four other cancer cell lines. Sarcoma-180 cells (5×10^6) were inoculated in the intraperitoneal cavity of ICR mice. β-Glucan (250 μg/mouse) was administered intraperitoneally every other day (7 times). Life span clearly increased in the DMJ-E-treated groups, compared with the nontreated groups. Previous reports showed that β-glucans were not toxic for tumors but played a role in stimulating host defense mechanisms [2]. β-Glucans are found in various species such as fungi,

yeast, algae, bacteria, and higher plants. β-Glucans are biological response modifiers that regulate host immune responses [6, 33] and have been promising molecules for cancer immunotherapy [3, 7, 24, 29]. In our experiments, the cytotoxic response depends on the cell types and its dosage, although the mechanism by which it downregulates cell proliferation remains to be clearly resolved. Our findings are similar to those of previous observations on the use of β-glucans in enhancing immunological functions. All of these activities strongly suggested that DMJ-E is a good candidate as an immune-modulating agent.

Acknowledgments

This work was approved by the ethics committees of both Konkuk University and Dankook University Hospital and with the informed consent of all subjects involved in this study. We are grateful to Dr. Shin-Sook Yoon of Kangnam University for conducting various molecular structure analyses with our samples, and to Dr. JongWan Kim of Dankook University Hospital for his supplying PBMCs. This work was supported by the faculty research fund of Konkuk University in 2006.

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