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Ginsan Improved Th1 Immune Response Inhibited by Gamma Radiation

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Gamma radiation causes suppression of the immune function, and immune properties are related to cytokine production. In the present study, the polysaccharide, Ginsan, purified from an ethanol-insoluble fraction of Ginseng (Panax ginseng C.A. Meyer, Araliaceae) water extract was studied to assess its effects on the immunosuppressive activities of gamma radiation. Ginsan was found to stimulate murine normal splenocytes by inducing the mRNA expressions of Th1 and Th2 type cytokines, and also restore the mRNA expression of IFN-γ, Th1 cytokine, after its inhibition by whole-body gamma irradiation. Therefore, Ginsan was found to restore the T lymphocytes function that had been suppressed by gamma irradiation in allogenic MLR (mixed lymphocyte reactions). However, Ginsan exhibited no excessive stimulatory effects on the control group. The above results indicated that Ginsan may constitute a new noble agent for the improvement of gamma radiation-induced immunosuppression.

Key words: Ginsan, Gamma radiation, Cytokines

INTRODUCTION

Recently, the discovery of new noble agents has been extensively pursued in order to find a way to reduce the deleterious side effects of radiation. Many cytokines, in particular, have been purified, characterized, cloned and synthesized. Recombinant cytokines are well known for their function as immune modulators (Geimm *et al.*, 1982; Mule *et al.*, 1985; Rosenberg *et al.*, 1985; Tang *et al.*, 1986; Ballas *et al.*, 1987; Ochoa *et al.*, 1987), and have been used in immunotherapy for a vast variety of cancers, but tend to be too toxic, with very short half lives, suggesting that their endogenous induction may prove a more effective immunotherapeutic approach in the treatment of cancer. Therefore, immune modulators able to induce and prolong the secretion of various endogenous cytokines are expected to be quite useful in prevention and treatment of cancer (Oldham 1982; Talmadge *et al.*, 1984; Smalley and Oldham 1986; Talmadge and Herberman 1986; Wimer 1989). Indeed, biological response modifiers (BRM), including bacillus calmette-Guerin (BCG) (Yanagawa *et al.*, 1979), OK-432 (Uchida and Micksche 1981), IFN (Murren and Buzaid 1989, Strander 1989), AS101 (Sredi *et al.*, 1987), lentinan (Maeda *et al.*, 1984) and flavonoids (Wilrout and Hornung 1988) have been identified as nontoxic immune modulators and shown to act as effectors or mediators of effective immune responses in cancer prevention and therapy.

Ginseng (*Panax ginseng* C.A. Meyer, *Araliaceae*) has been studied for a very long time. Its anticancer activities appear to be due to the direct cytotoxic effects of low molecular-weight poly-acetylene compounds (Kim *et al.*, 1989) and the immune modulating activity of high molecular-weight compounds (Singh *et al.*, 1983, Gao *et al.*, 1989, Kim *et al.*, 1990). It has been reported that ginseng polysaccharides can induce natural killer (NK) cell activity, lymphocyte proliferation and macrophage activation. Therefore, in a previous study, we purified and characterized polysaccharide Ginsan from old white Ginseng, and found Ginsan to be an excellent radioprotective agent, as well as an ideal BRM. Ginsan was demonstrated to induce the proliferation of normal

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splenocytes and a number of GM-CFU in normal bone marrow cells, both *in vitro* and *in vivo*. Ginsan also improved various hematopoietic parameters, including bone marrow and spleen cellularity, GM-CFU content and CFU-S levels in bone marrow (Song *et al.*, 2003).

The immunosuppressive effect of gamma radiation is well known. Different levels of radiosensitivity have been reported among lymphocyte subpopulations; for example, Th1 cells are more radiosensitive than Th2 cells (Galdiero *et al.*, 1994). We recently reported that gamma radiation inhibited the mRNA expression of IFN-γ, a Th1 cytokine, as well as related transcription factors, while Th2 cytokine and proinflammatory cytokine expressions were rapidly induced on exposure to gamma radiation. The gamma-induced inhibition of Th1 cytokine mRNA expression also effected a reduction in the functional activity of T lymphocytes (Han *et al.*, 2002), which resulted from the effects of gamma radiation, rather than indicating differential radiosensitivities among lymphocyte subpopulations. In order to improve these immunosuppressive effects of gamma radiation, in this study, a new noble agent, Ginsan, was investigated for its effect on the mRNA expression of cytokines in murine splenocytes *in vitro* and *in vivo*, as well as the functional activity of the T lymphocytes, using allogenic MLR. Ginsan was found to be a good candidate for the alleviation of gamma radiation-induced immunosuppression.

MATERIALS AND METHODS

Animals

Seven or eight-week old Balb/c and C57BL/6 mice were maintained in an accredited Laboratory Animal Care facility operated by the Korea Cancer Center Hospital (KCCH). We used 2~4 mice per group in every sample preparation. All mice used, weighing 20±2 g each and were supplied with solid feed and water. The temperature of the laboratory in which the animals were maintained was 22 ± 2°C, with a humidity of 55~60%.

Preparation of Ginsan

Polysaccharide 'Ginsan' was purified from an ethanol-insoluble fraction of a *Panax ginseng* water extract, as described previously (Yun *et al.*, 1993). Further purification was performed by Sephacryl S-500 gel column chromatography (2.6×100 cm), with 0.1 M sodium phosphate buffer (pH 7.4) and by DEAE-Sephadex A50 column chromatography (3×30 cm), with a 0 to 1 M linear gradient of NaCl. The Ginsan was composed of α (1→6) glucopyranoside and β (2→6) fructofuranoside, with a molar ratio of 3:7, and average molecular weight of 2,000 Kd (Data are in press). The lot containing high LAK activity and splenocyte proliferation was used in the following experiments.

Cell culture

The spleens were removed from the mice by sectioning with sterilized scissor. The extracted spleens were transferred to fresh RPMI 1640 medium, containing 2×10^{-2} M HEPES, 2×10^{-3} M glutamine, 1×10^{-3} M pyruvate, 100 U/mL penicillin, 50 μg/mL streptomycin, 5×10^{-5} M 2-mercapto-ethanol and 1% non-essential amino acids, supplemented with 5% fetal bovine serum (FBS) (Life Technologies, Cergy-Pontoise, France), and then rinsed. The spleens were then pressed and chopped gently with the tail of a 1 mL syringe. The cell suspension was filtered through sterilized gauze in order to remove cell debris, washed with fresh RPMI 1640 medium and the cells counted using the trypan blue exclusion method. The isolated splenocytes were cultured in RPMI 1640 medium and treated with Ginsan (50 μg/mL) for 3, 6 and 24 h. The Ginsan (100 mg/kg) solutions were injected intraperitoneally (i.p.) into mice 24 h prior to whole-body irradiation. The concentration of Ginsan was determined in our previous report (Song *et al.*, 2003). The irradiated mice were maintained in the animal care laboratory of the KCCH for a further 5 days, and then sacrificed. The splenocytes were isolated from the mice and cultured in RPMI 1640.

Gamma irradiation

The splenocytes were irradiated with 5 Gy of γ-radiation, and the mice with 4.5 Gy, at a dose rate of 97.1 cGy/min, using a ^{60}Co theraton-780 (Atomic Energy of Canada, Ltd., Canada).

RNA preparation

Total RNA was extracted as follows. After appropriate treatments and incubation, the cells were collected and disrupted with RNAzol (Tel-Test, Inc. Texas, USA). Fifty microliters of chloroform were added and the mixture vortexed and incubated on ice for 10 minutes. The mixture was centrifuged at 12,000 rpm for 15 minutes at 4°C, and the upper layer transferred to a new chilled tube. An equal volume of chilled isopropanol was added to the upper layer, and incubated for 1 h at -20°C. After 20 minutes of centrifugation at 12,000 rpm at 4°C, the RNA pellets were collected and washed with chilled 75% ethanol. The RNA pellets were completely dried at room temperature, and dissolved in diethyl pyrocarbonate (DEPC)-treated distilled water. The RNA concentrations were determined spectrophotometrically at a wavelength of 260 nm.

Semi-quantitative reverse transcriptase (RT)-polymerase chain reaction (PCR)

One microgram of intact total RNA was reversibly

transcribed into first-strand cDNA, and the transcribed cDNA amplified using a polymerase chain reaction (PCR), as follows. A final volume of 20 μL reverse transcriptase (RT) reaction mixture contained: 50 mM Tris-HCl (pH 8.3), 3 mM $MgCl_2$, 75 mM KCl, 2.5 μg/mL $pd(N)_6$ primer (random hexamer), deoxyribonucleoside tri-phosphate (dNTP) mixture (0.5 mM each of 2' deoxyadenosine 5'-triphosphate (dATP), 2' deoxyguanosine 5'-triphosphate (dGTP), 2' deoxythymidine 5'-triphosphate (dTTP), 2' deoxycytosine 5'-triphosphate (dCTP) and 10 U of AMV-reverse transcriptase (RT). The RT reaction was performed at 60°C for 1 minute, 37°C for 90 minutes and at 95°C for 1 minute, and the synthesized cDNA then cooled on ice. The reaction mixture for PCR contained: 10 μL cDNA template from the RT reaction, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, dNTP mixture (0.5 mM each of dATP, dGTP, dTTP and dCTP), 1.0 μM of each primer and 0.5U Taq DNA polymerase. The oligonucleotide primers used in these experiments are listed in Table I. PCR was performed with a DNA thermal cycler (Hybaid, United Kingdom) at 94°C for 1 minute, at 55~60°C for 1 minute and at 72°C for 1 minute per cycle. The amplified products were visualized by electrophoresis on a 1% agarose gel in the presence of 0.5 μg/mL ethidium bromide (EtBr). β-Actin primers were used as an internal control in all cases.

RNase protection assay (RPA)

The Ginsan (100 mg/kg) was administered to mice 24 h prior to irradiation, and the mice kept alive for a further 5 days. Subsequently, the total RNA was isolated from the splenocytes, and frozen at -70°C until used. RT-PCR, as described above, and RPA were performed. A cytokine RNA probe, with a maximum yield of 3×10^6 Cherenkov counts/μL, was synthesized *via* multi-probe DNA templates, following the manufacturer's instructions (Pharmingen Co.). The total RNA (10 or 20 mg) in DEPC-treated distilled water was frozen for 15 minutes at -70°C, and then dried completely in a vacuum evaporator centrifuge without heating. The hybridization was then performed according to the manufacturer's instructions. The hybridized RNA samples were digested with 100 μL of RNase cocktail for 45 minutes at 30°C, and then purified. Ammonium acetate (4M, 120 μL) and 650 μL ice-cold 100% ethanol were added, and then precipitated at -70°C. The dried pellets were dissolved in 5 μL of 1X loading buffer, heated for 3 minutes at 90°C, and then cooled immediately prior to loading the samples onto the gel. A set of gel plates were cleaned with chloroform ($CHCl_3$) or DEPC treated water, and the gel mold (0.4 mm spacers) then carefully assembled. The glass plates were wiped with 70% ethanol and coated with SIGMACOAT. Five-percent acrylamide gel (19:1 acrylamide/bis) electrophoresis was then performed.

Table I. Oligonucleotides used in the RT-PCR

oligonucleotides		Sequence	Expected size
IFN-γ	5'-primer	5'-TACTGCCACGGCACAGTCATTGAA-3'	405
	3'-primer	5'-GCAGCGACTCCTTTTCCGCTTCCT-3'	
IL-2	5'-primer	5'-GTCAACAGCGCACCCACTTCAAGC-3'	451
	3'-primer	5'-GCTTGTTGAGATGATGCTTTGACA-3'	
IL-12 (p35)	5'-primer	5'-ACCTCAGTTTGGCCAGGGTC-3'	500
	3'-primer	5'-GTCACGACGCGGGTGGTGAAG-3'	
IL-4	5'-primer	5'-ACGGAGATGGATGTGCCAAACGTC-3'	279
	3'-primer	5'-CGAGTAATCCATTTGCATGATGC-3'	
IL-5	5'-primer	5'-ATGACTGTGCCTCTGTGCCTGGAGC-3'	243
	3'-primer	5'-CTGTTTTTCCTGGAGTAAACTGGGG-3'	
IL-10	5'-primer	5'-AGACTTTCTTTCAAACAAAGGACCAGCTGGA-3'	
	3'-primer	5'-CCTGGAGTCCAGCAGACTCAATACACACTGC-3'	421
IL-1β	5'-primer	5'-TGAAGGGCTGCTTCCAAACCTTTGACC-3'	361
	3'-primer	5'-TGTCCATTGAGGTGGAGAGCTTTCAGC-3'	
TNF-α	5'-primer	5'-GCGACGTGGAACTGGCAGAAG-3'	340
	3'-primer	5'-TCCATGCCGTTGGCCAGGAGG-3'	
GM-CSF	5'-primer	5'-CCCATCACTGTCACCCGGCCTTGG-3'	279
	3'-primer	5'-GTCCGTTTCCGGAGTTGGGGGGC-3	
β-actin	5'-primer	5'-TGGAATCCTGTGGCATCCATGAAAC-3'	349
	3'-primer	5'-TAAAACGCAGCTCAGTAACAGTCCG-3'	

Allogenic mixed lymphocyte reaction (MLR)

Six-week-old Balb/c mice (H-2^d) were treated with Ginsan (100 mg/kg) 24 h, or left untreated prior to the gamma-irradiation at a dose of 4.5 Gy. After 5 days, the mice were sacrificed, the splenocytes collected from each group, and used as responder cells. Concomitantly, the splenocytes from the C57BL/6 mice (H-2^b) were irradiated with a dose of 20 Gy, and used as stimulator cells. Responder cells (5×10^6 cells/mL) were cultured in RPMI 1640, supplemented with 5% (v/v) fetal bovine serum (FBS), 2×10^{-2} M HEPES buffer, 2×10^{-3} M glutamine, 1×10^{-3} M sodium pyruvate, 100 U/mL penicillin, 50 μg/mL streptomycin, 5×10^{-5} M 2-mercaptoethanol and 1% non-essential amino acids, together with stimulator cells (5×10^6 cells/mL), on flat-bottomed 96-well microplates, for 4 days at 37°C, in an atmosphere of 5% CO_2. Each experiment was performed in triplicate. The control contained only responder cells. ^{3}[H]-Thymidine (2 mCi/well) was added to each well 24 h prior to harvesting, and the amount of ^{3}[H]-thymidine incorporated determined using a β-counter (Tri-Carb 4530, Packard Co.).

RESULTS

Ginsan induced the mRNA expression of cytokines *in vitro*

In order to assess the effects of Ginsan on cytokine gene regulation *in vitro*, the splenocytes isolated from Balb/c mice were cultured for 3, 6 or 24 h, either in the presence or absence of 50 μg/mL Ginsan. RT-PCR was performed on the total extracted RNA to determine the mRNA levels of Th1 type, Th2 type and proinflammatory cytokines. As shown in Fig 1, IFN-γ was induced after 6 h, but reduced 24 h after Ginsan treatment. Conversely, the mRNA level of IL-2 was highly induced at 24 h, whereas that of IL-12 was found to be significantly elevated at all times. Th2 type cytokines, such as IL-4, IL-5 and IL-10, were rapidly induced within 3 h, and persisted for 6 or 24 h in the presence of Ginsan. The IL-5 levels were increased at 6 hours, and persisted until the 24 h measurement (Fig. 2). mRNA expression of the proinflammatory cytokines also increased very rapidly, and persisted until the 24 h measurement (Fig. 3). The above results strongly indicated that Ginsan was able to activate the immune cells through the induction of endogenous cytokine expression.

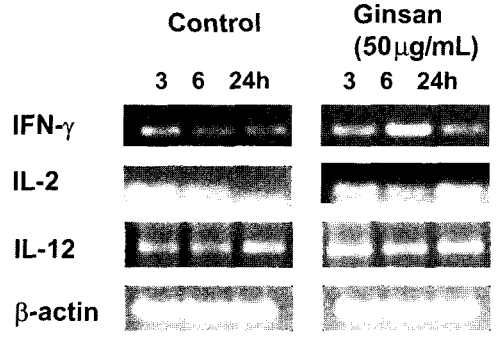

Fig. 1. Effects of Ginsan on mRNA expressions of Th1 type cytokines *in vitro*. RT-PCR was performed with total RNA isolated from untreated cells (control), and cells cultured for the indicated times in the presence of Ginsan (50 μg/mL). One microgram of intact total RNA was reversibly transcribed into first strand cDNA, and amplified using a polymerase chain reaction (PCR). The PCR products were analyzed by 1% agarose gel electrophoresis. The data shown represents one of two independent experiments.

Ginsan prevented the inhibition of IFN-γ mRNA expression by gamma irradiation *in vivo*

Ginsan solution or PBS (control) was injected intraperitoneally (*i.p.*) into groups of three or four mice, 24 h prior to gamma irradiation. Five days after irradiation, the

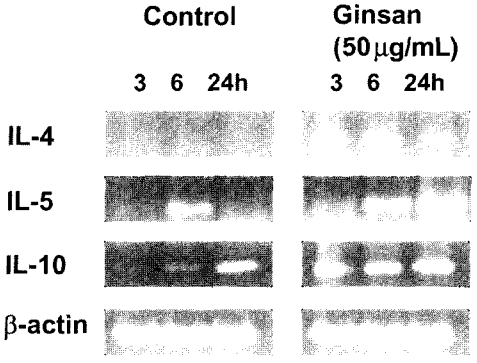

Fig. 2. Effects of Ginsan on mRNA expression of Th2 type cytokines *in vitro*. RT-PCR was performed with total RNA isolated from untreated cells (control), and cells cultured for the indicated times in the presence of Ginsan (50 μg/mL). Isolation of total RNA, its transcription into cDNA and amplification by PCR were carried out, as described in the legend to Fig. 1 and the methods section.

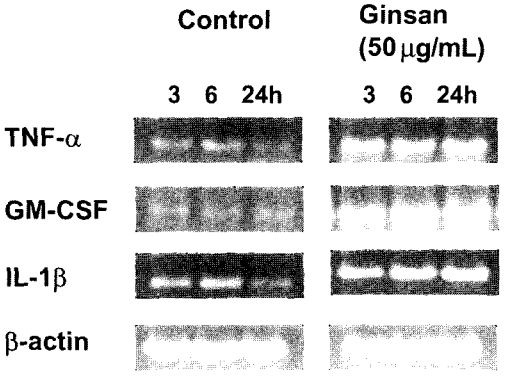

Fig. 3. Effects of Ginsan on mRNA expression of proinflammatory cytokines *in vitro*. RT-PCR was performed with total RNA from untreated cells (control), and cells cultured for the indicated times in the presence of Ginsan (50 μg/mL). Isolation of total RNA, its transcription into cDNA and amplification by PCR were carried out, as described in the legend to Fig. 1 and the methods section.

splenocytes were isolated, the total RNA purified, and RT-PCR and RPA performed, as described above. We confirmed our previous, recently-reported RT-PCR data (Song *et al.*, 2003). The IFN-γ levels were reduced as a result of irradiation, but recovered upon Ginsan treatment (Fig. 4). We performed RNase protection assays (RPA) to verify these effects of Ginsan on the cytokine regulation *in vivo*. As shown in Fig. 5, we also observed gamma radiation-induced reductions in the levels of *IL-4*, *IL-10* and IFN-γ cytokine expressions. The protection of the IFN-γ levels due to Ginsan treatment was detected, but IL-4 and IL-10 appeared not to be protected by Ginsan. The results of RPA were consistent with our RT-PCR data. Interestingly, IL-15, which is involved in proliferation and cytokine production in T cells, was detected, and slightly increased as a result of Ginsan treatment. The above results indicated that Ginsan was able, *in vivo*, to reverse or mitigate the gamma-induced reduction in the levels of Th1 cytokines.

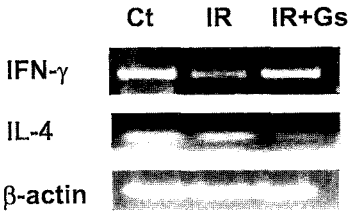

Fig. 4. Effects of Ginsan on cytokine expression examined by RT-PCR. Ginsan (100 mg/kg) and PBS solution were injected intraperitoneally (i.p.) into mice 24 h prior to whole-body gamma irradiation (Ct: control group, IR: irradiated group, IR+Gs: treated with Ginsan and irradiated group). Five days after irradiation, the mice were sacrificed, the splenocytes isolated and the total RNA extracted from each group. RT-PCR was performed as described in the legend to Fig. 1 and the methods section.

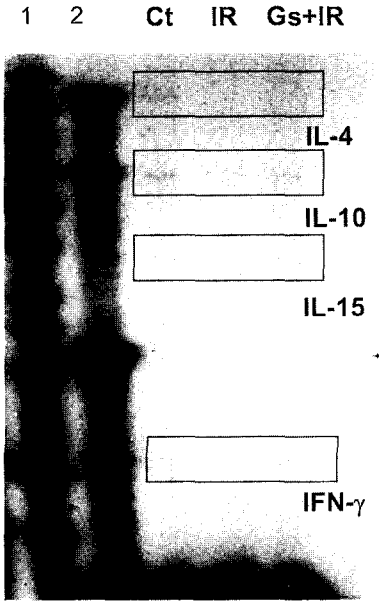

Fig. 5. Effects of Ginsan on cytokine expression examined by RNase protection assay (RPA). Ginsan (100 mg/kg) and PBS solutions were injected intraperitoneally (i.p.) into mice 24 h prior to whole-body gamma irradiation (Ct: control group, IR: irradiated group, IR+Gs: treated with Ginsan and irradiated group). Five days after irradiation, the mice were sacrificed and the splenocytes isolated from each group. The total RNA (10~20 μg) extracted from each group was hybridized with a synthesized RNA probe, and the hybridized RNA digested with RNase. Digested samples were analyzed by 5% acrylamide gel (19:1 acrylamide/bis) electrophoresis (Lane 1; undigested probe used as marker, lane 2; control RNA supplied from Pharmingen).

Ginsan protected the functional activity of T lymphocytes inhibited by gamma irradiation

The gamma radiation-induced reduction of Th1 type cytokines can result in the inhibition of Th1 immune responses, as the reduction of Th1 type cytokines affects the differentiation of Th1 cells. In order to evaluate the functional consequences of T-lymphocytes by gamma irradiation and the treatment with Ginsan, allogenic MLR was performed, as described in the Methods section. Thus, Ginsan (100 mg/kg) was injected intraperitoneally (i.p.) into mice 24 hours prior to gamma irradiation, and the splenocytes isolated from each group (Balb/c; H-2^d), 5 days later, for use as responder cells. They were cultured for 4 days, with prepared stimulators (C57BL/6; $H2^b$) at a

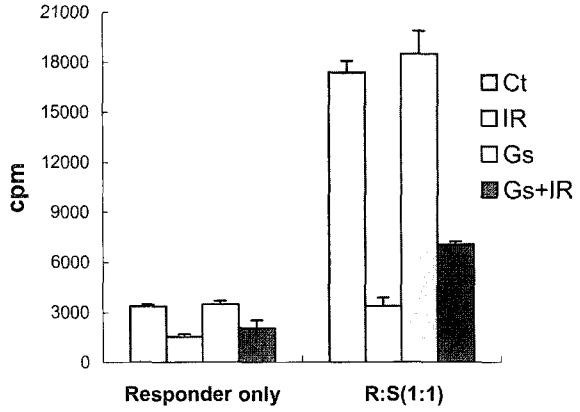

Fig. 6. Effects of gamma irradiation and Ginsan on the functional activity of T lymphocytes. Ginsan (100 mg/mL) and PBS solutions were injected intraperitoneally (i.p.) into mice 24 h prior to whole-body gamma irradiation (Ct: control group, IR: irradiated group, Gs: group treated with Ginsan, Gs+IR: group treated with Ginsan and irradiated). Five days after irradiation, the mice of each group were sacrificed and the splenocytes isolated. The splenocytes of each group were used as responder cells (Balb/c: H-2^d) and cultured in the presence of prepared stimulator cells (C57BL/6: H-2^b), which had been irradiated with 20 Gy, at a ratio of 1:1 (responder: stimulator), in flat-bottomed 96-well microplates for 4 days. ^{3}[H]-thymidine (2 μCi/well) was added to each well 24 h prior to harvesting, and the amount of ^{3}[H]-thymidine incorporated measured by a β-counter. This experiment was carried out in triplicate from three different preparations. The values are presented as the mean ± SE.

1:1 ratio (responder: stimulator), in flat-bottomed 96-well microplates. As shown in Fig. 6, Ginsan (Gs) did not induce excess activity in the control group (Ct), indicating that Ginsan might be a good candidate as an immune modulator. While the activity of the irradiated group (IR) was 20~22% that of the control group (Ct), the activity of the treated group (Gs + IR) recovered to a level 40% that of the control group. This represents a 2-fold increase compared with the activity of the T cells in the irradiated group (IR). Based on the recovery of Th1 cytokines, Ginsan treatment can safely be said to have improved the effective function of the T cells.

DISCUSSION

It is well established that gamma radiation causes immunosuppression and inflammation. We recently reported that gamma radiation induced immunosuppression by inhibiting the mRNA expression of Th1 type cytokines and related transcription factors. We also determined that gamma radiation interfered with STAT1, which is involved in the IFN-γ signaling pathway (Han *et al.*, 2002). Therefore, we have been searching for new agents that can improve the gamma radiation-induced reduction of Th1 type cytokines. Indeed, new noble agents are currently being actively sought by many researchers, in the hope of finding a way to reduce the deleterious side effects of radiation. The induction of endogenous cytokines appears to be a more promising approach to cancer immunotherapy than recombinant cytokines. Therefore, biological response modifiers (BRM) are used as nontoxic immune modulators, effectors or mediators of an effective immune response in the field of cancer prevention and therapy.

We selected Ginsan, a polysaccharide extracted from *Ginseng Radix Alba*, as a likely candidate for the relief of the immunosuppressive effects of gamma irradiation. The radioprotective and immune modulating effects of Ginsan were recently demonstrated in our laboratory (Song *et al.*, 2003): Ginsan effectively improved various reduced hematopoietic parameters, including bone marrow and spleen cellularity, which had been compromised by gamma radiation. In the present study, we demonstrated that Ginsan induced the production of Th1, Th2 and proinflammatory cytokines in normal splenocytes *in vitro* (Fig. 1~3), further confirming our previous results, which showed that Ginsan was a mitogen of T lymphocytes (Lee *et al.*, 1997).

In order to assess the *in vivo* effects of Ginsan on cytokine production after gamma irradiation, we administered Ginsan to mice 24 h before whole-body gamma irradiation. The duration of administration is very important; therefore, we previously established the optimal conditions for the administration of Ginsan. For examination of the cytokine profiles, the mice were sacrificed 5 days after irradiation, when the spleen cell number had begun to recover (Song *et al.*, 2003). Although a reduction in the levels of Th1 and Th2 type cytokines was observed 5 days post-irradiation, Ginsan mainly induced the expression of Th1 type cytokines, such as IFN-γ, in both RT-PCR and RPA data (Figs. 4, 5). Conversely, the levels of IL-4 and IL-10, both Th2 type cytokines, were found to have only slightly increased above those in the radiation/Ginsan group, and were not comparable to the RPA data of the control group. Moreover, IL-4 was not induced by Ginsan treatment, according to our RT-PCR data, but Ginsan clearly stimulated IFN-γ mRNA expression.

As the generation of cytotoxic T lymphocytes (CTL) by Th1 type cytokines, such as IFN-γ and IL-12, is crucial in a host's response to tumors, transplants and viruses, and is particularly related to the mixed lymphocyte reaction (MLR), allogenic MLR was performed. As shown in Fig. 5, Ginsan-mediated protection of the Th1 type cytokine expression resulted in an improvement in the functional activity of the T lymphocytes. Gamma irradiation is known to significantly inhibit the proliferation of effective T cells by reducing the levels of Th1 type cytokines. However, Ginsan treatment prior to irradiation prevented this gamma radiation-induced effect, *via* the induction of endogenous cytokine gene expression. Interestingly, IL-15, which is associated with proliferation and cytokine-mediated T cell production, was detected in our RPA data, indicating that Ginsan has a slight inductive effect on IL-15 (Fig. 5). IL-15 exhibits similar activity with IL-2, possibly due to its interaction with IL-2 receptors. However, it is becoming evident that IL-15 and IL-2 differentially exert their effects on a number of cell types, and are thus expected to play distinct roles *in vivo* (Shuat *et al.*, 1994). It has recently been reported that IL-15 increases the activity of cytotoxic T lymphocytes (Khan and Casciotti 2000; Chen *et al.*, 2000). Therefore, increased IL-15 and IFN-γ mRNA expressions, due to Ginsan treatment, are expected to contribute to improvements in the gamma radiation-inhibited functional activity of T lymphocytes. Although Ginsan induced mRNA expressions of all cytokine types in normal splenocytes *in vitro*, allogenic MLR revealed no excessive stimulatory effects of Ginsan in the Ginsan-treated control group (G). If Ginsan excessively activates the normal immune system, it would be practically unsuitable in its application as an immune modulator. Furthermore, Ginsan enhanced the effective functional activity of the T lymphocytes, about 2 folds that in the irradiated group without Ginsan (IR) (Fig. 6), thus indicating that Ginsan effectively alleviates the gamma radiation-induced inhibition of Th1 type cytokine expression, and improves the gamma-induced inhibition of the Th1 immune response.

In summary, Ginsan may constitute a potent stimulator,

as it clearly stimulates the endogenous mRNA expression of cytokines. IFN-γ and Th1 type cytokines, were particularly effectively protected against gamma irradiation. Ginsan treatment also clearly enhanced the functional activity of T cells. Therefore, Ginsan should be considered as a good candidate for improvement of gamma radiation's immunosuppressive effects.

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