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Gamma Irradiation-reduced IFN-γ Expression, STAT1 Signals, and Cell-mediated Immunity

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The *signal* transducer and activator of transcription (STAT)1 is a cytoplasmic-transcription factor that is phosphorylated by Janus kinases (Jak) in response to interferon γ (IFN-γ). The phosphorylated STAT1 translocates to the nucleus, where it turns on specific sets of IFN-γ-inducible genes, such as the interferon regulatory factor (IRF)-1. We show here that gamma irradiation reduces the IFN-γ mRNA expression. The inhibition of the STAT1 phosphorylation and the IRF-1 expression by gamma irradiation was also observed. In contrast, the mRNA levels of IL-5 and transcription factor GATA-3 were slightly induced by gamma irradiation when compared to the non-irradiated sample. Furthermore, we detected the inhibition of cell-mediated immunity by gamma irradiation in the allogenic-mixed lymphocytes' reaction (MLR). These results postulate that gamma irradiation induces the polarized-Th2 response and interferes with STAT1 signals, thereby causing the immunosuppression of the Th1 response.

Keywords: Gamma-irradiation, IFN-γ, STAT 1

Introduction

T helper (Th) cells can be divided into two types, depending on the secreted-cytokine patterns. Th1 cells secrete interleukin-2 (IL-2), interferon-γ (IFN-γ), and interleukin-12 (IL-12). They can activate macrophage and serve as mediators of cell-mediated immune responses, such as delayed-type hypersensitivity (DTH), and promote tumoricidal activity. In contrast, Th2 cells secrete interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), and interleukin-10 (IL-10). They can induce IgE- and eosinophil-mediated reactions by the production of cytokines, and promote humoral immunity (Tepper *et al*., 1992; Aruga *et al*., 1997). Th1 and Th2 cells reciprocally inhibit the growth and function of the other cell type. IFN-γ is a pleiotropic cytokine with antiproliferative and immunomodulatory activities that are crucial for the regulation of immune responses (Farrar and Schreiber, 1993). IFN-γ signaling is initiated by IFN-γ binding to its receptor and inducing receptor dimerization. The receptor-associated Jak1 and Jak2 are phosphorylated, which then results in kinase activation. The cytoplasmic domain of the receptor is phosphorylated by the activated kinase, which recruits the signal transducer and activator of transcription (STAT) 1 (Greenlund *et al*., 1994, 1995; Sakatsume *et al*., 1995), as well as its activation by tyrosine phosphorylation, which leads to its dimerization. The dimerized STAT1 translocates to the nucleus, where it activates the transcription of IFN-γ-responsive genes, such as IRF-1 (interferon regulatory factor-1) (Harada *et al*., 1989). IRF is a transcription factor of interferon-related genes that can induce the effective differentiation of Th1 cells (Taki *et al*., 1997; Coccia *et al*., 1999). GATA-3, a transcription factor that is selectively expressed on naive and Th2 cells, inhibits Th1 development and modulates IL-4 and IL-5 production (Zhang *et al*., 1997); it also inhibits IFN-γ production (Ouyang *et al*., 1998). In antitumor immunity, the generation of cytotoxic T lymphocytes (CTL) by Th1 cytokine IFN-γ is essential since antitumor-immunity is exerted by the activation of the Th1 response.

UV light is known for its immunosuppressive properties, which are demonstrated by the inhibition of the cellular-immune response (Kripke 1990; Chapman *et al*., 1995). It interferes with Th1 type cytokine production, such as IFN-γ and IL-7, and their signaling pathways on murine keratinocytes (Aragane *et al*., 1997; Yoshimori *et al*., 1997). The immunosuppressive effect of gamma irradiation is also well known, but its mechanism has not been examined. Since Th1 cytokines are important in cellular immunity, the inhibition of its expression by gamma irradiation may

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contribute to immunosuppressive effects. In the present study, we report that the IFN-γ mRNA expression is reduced by gamma radiation. STAT1 activation is also interfered by gamma irradiation; the expression of IRF-1 mRNA decreased. However, the relative expression of IL-5 and GATA-3 that was related to the Th2 response is slightly induced. We also observed that cellular immunity, as measured by MLR, is reduced by gamma irradiation. These results suggest that gamma irradiation-mediated immunosuppression could be due to the down regulation of the Th1 type cytokine.

Materials and Methods

Animals Seven-week-old Balb/c female mice were maintained in an accredited Laboratory Animal Care facility that is operated by the Korea Cancer Center Hospital (KCCH).

Cell culture The splenocytes that were isolated from Balb/C mice were cultured in a RPMI 1640 medium that contained 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-mercaptoethanol, and 1% non-essential amino acids that were supplemented with 5% fetal bovine serum (FBS) (Life Technologies, Cergy-Pontoise, France). Five milliliters of cell suspension (2×10^6 cells/ml) were plated in 6-well plates. The cells were counted by the trypan-blue exclusion method. For T cell activation, the splenocytes were stimulated with 2.5 μg/ml of concanavalin A (Con A) at 3 h after gamma irradiation, then incubated another 3 h. The stimulated splenocytes were used for an analysis of the Th1 type cytokine expression by reverse transcriptase (RT)-polymerase chain reaction (PCR). For the IL-5 expression analysis, the splenocytes were stimulated with 2.5 μg/ml of Con A 5 min after gamma irradiation, then incubated for 1, 6, and 24 h, respectively. The splenocytes were stimulated for 2 days before gamma irradiation for a GATA-3 expression analysis. Total RNAs from the non-irradiated cells at each time point were used as controls.

Gamma irradiation Throughout the study, the splenocytes were irradiated at a dose of 5 Gy of γ-irradiation using a ^{60}Co theraton-780 (Atomic Energy of Canada, Ltd., Ontario, Canada) at a dose rate of 1.394 Gy/min.

Cell-viability assay Cell viability was examined in a flat-bottomed 96-well microplate using MTT reagents (Yi *et al*., 2001). Splenocytes suspension (150 μl), which was irradiated at a dose of 1, 3, 5 Gy or none, was plated in a flat-bottomed 96-well microplate at a density of 5×10^6 cells/ml and cultured for 2, 3, and 5 days at 37°C, 5% CO_2. At the end of the culture, 15 μl of a MTT (3-[4,5-dimethylthiozol-2yl]-2,5-diphenyltetrazolium) dye solution (5 mg/ml in PBS) was added to each well, and the plate was incubated for another 4 h. One hundred microliters of 0.04 N-HCl/isopropanol were added to each well. The wells were mixed well with a pipette to dissolve the dark blue MTT-formazane crystals. The absorbance was measured at 540 nm using a Behring ELISA processor II (Biotrin Intl., Lyon, France).

Semi-quantitative RT-PCR After appropriate treatments and incubation for varying time periods, the cells were collected and washed with PBS. Total RNA was extracted from the irradiated or non-irradiated splenocytes by lysis in guanidinium isothiocyanate using RNAzol (Tel-Test, Inc., Friendswood, USA). The RNA concentrations were determined spectrophotometrically at the absorbance of 260 nm. One microgram of intact total RNA was reversibly transcribed into first-strand cDNA, which was then amplified using PCR. The final volume of the 20 ml 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), dNTP mixture (0.5 mM each of dATP, dGTP, dTTP, and dCTP), and 10 U of AMV-reverse transcriptase (RT). The reaction mixture for PCR contained 10 ml cDNA templates from the RT reaction as follows: 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 mM of each primer, and 0.5 U Taq DNA polymerase. The oligonucleotide primers that were used in these experiments are listed in Table 1. PCR was performed with a DNA thermal cycle (Hybaid, Middlesex, UK) at 94°C for 1 min, at 55~60°C for 1 min, and at 72°C for 1 min per cycle. The amplified products were visualized by electrophoresis on a 1% agarose gel in the presence of 0.5 μg/ml ethidium bromide. In all of the cases, the b-Actin primers were used as the internal control.

Immunoprecipitation and Western blotting For the immunoprecipitation and Western blot analysis (Yi *et al*., 2001), the cells were stimulated with 2.5 μg/ml of Con A for 2 or 3 days prior

Table 1. Oligonucleotides primers used in RT-PCR

oligonuleotides		Sequence	Expected size
IFN-γ	5'-primer	5'-TACTGCCACGGCACAGTCATTGAA-3'	405
	3'-primer	5'-GCAGCGACTCCTTTTCCGCTTCCT-3'	
IL-5	5'-primer	5'-ATGACTGTGCCTCTGTGCCTGGAGC-3'	243
	3'-primer	5'-CTGTTTTTCCTGGAGTAAACTGGGG-3'	
GATA-3	5'-primer	5'-CTTATCAAGCCCAAGCGAAG-3'	505
	3'-primer	5'-TAGAAGGGGTCGGAGGAACT-3'	
IRF-1	5'-primer	5'-AGGGCTTAGGAGGCAGAGTC-3'	500
	3'-primer	5'-AAAGGCCTAGACTGGGGAGA-3'	
β-actin	5'-primer	5'-TGGAATCCTGTGGCATCCATGAAAC-3'	349
	3'-primer	5'-TAAAACGCAGCTCAGTAACAGTCCG-3'	

to gamma irradiation. Recombinant IFN-γ (each 10 ng/ml) was added to the cell suspension just after gamma irradiation. The cells were harvested on ice within 15 min of gamma irradiation. The cells were lysed in a TNN lysis buffer (40 mM Tris-HCl, 120 mM NaCl, 0.1% Nonidet-P40) that contained proteases and phosphatase inhibitors (20 mM NaF, 20 mM β-glycerophosphate, 1 mM phenylmethyl-sulfonyl-fluoride, 500 mM sodium orthovanadate, aprotinin 2 μg/ml, leupeptin 10 μg/ml). The protein concentration in the cellular extracts was determined by the Bradford method. The lysates were immunoprecipitated with anti-Stat antibodies (source) that were diluted at the ratio of 1 : 1000. The immuno complex was separated on 7.5% SDS-PAGE and electro-transferred to the nitrocellulose membrane (Amersham Pharmacia Biotech, Buckinghamshire, UK). The immunoblots were blocked with a TNN buffer that contained 0.5% bovine serum albumin (BSA), protease, and phosphatase inhibitors for 1 h at room temperature. The blocked N/C membrane was probed with 1 μg/ml anti-phosphotyrosine antibody (PY20). The N/C membrane was washed and probed with a horseradish peroxidase-conjugated antibody (Ab) as a secondary antibody (1 μg/ml). An enhanced chemiluminescence (ECL) Western blotting analysis was used for detection of the antigen (Ag). The same blots were reprobed with anti-Stat (Stat1, 3, 4, 6) Abs (1 μg/ml) in order to confirm the total amount of Stat molecules.

Allogenic-mixed lymphocytes reaction (MLR) Seven week-old Balb/c mice (H-2^d) were irradiated with a dose of 4.5 Gy. After 5 days, the mice were sacrificed and splenocytes were collected, then used as responder cells. The splenocytes from the C57BL/6 mice (H-2^b) were irradiated with a dose of 20 Gy for stimulator cells. Responder cells (5×10^6 cells/ml) were cultured with stimulator cells (5×10^6 cells/ml) on a flat-bottomed 96-well microplate for 4 days at 37°C in 5% CO_2 in triplicate. The control was cultured without stimulator cells. At 24 h before harvest, ^{3}[H]-Thymidine (2 μCi/well) was added to each well. The amount of the incorporated ^{3}[H]-thymidine was determined using a β-counter (Tri-Carb 4530, Packard Co., Meriden, USA) (Cho *et al*., 2001).

Statistical analysis All of the experiments were carried out in triplicate from three or five different preparations. The values are presented as the mean ± SE. The statistical significance of differences between the groups was determined using a Student's t-test; p values less than 0.05 and 0.001 were considered significant.

Results

Effect of gamma irradiation on cell viability Preliminary experiments were carried out to draw a correlation curve between the gamma-irradiation dose and survival of the lymphocytes. The splenocytes that were isolated from mice were irradiated at 1, 3, and 5 Gy. The irradiated and non-irradiated cells were cultured 2 or 3 days, and the surviving cells were measured using a MTT assay. The results are shown in Figure 1. The survival of the irradiated cells at 5 Gy was reduced to 75% of the non-irradiated cells after 3 days. The same results were obtained by a trypan-blue exclusion assay (data not shown). Therefore, we selected 5Gy as a sublethal dose to conduct this study.

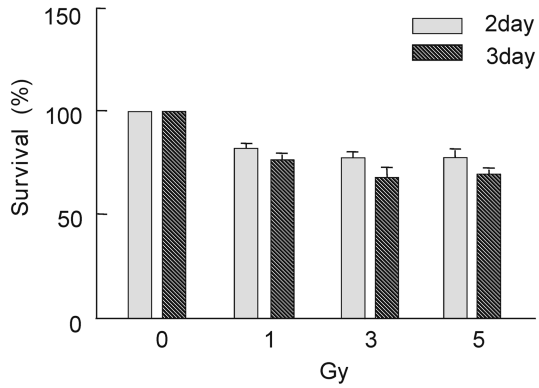

Fig. 1. Viability of splenocytes at varying doses of g-irradiation. Splenocyte suspensions that were isolated from Balb/c mice were cultured in a flat-bottomed 96-well plate at a density of 5×10^6 cells/ml for 2 or 3 days. Cell survival was measured by a MTT assay. These experiments were carried out in triplicate from three preparations. The values are presented as mean ± SE.

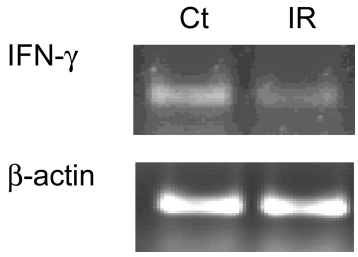

Fig. 2. Down-regulation of IFN-γ in irradiated splenocytes stimulated with Con A. The splenocytes were irradiated at the dose of 5 Gy and stimulated with Con A (2.5 μg/ml) 3 h after gamma irradiation. Total RNA from the cells was isolated after another 3 hr of incubation. After a semi-quantitative RT-PCR assay, the products from the untreated (Ct) and irradiated cells (IR) were analyzed on 1% agarose gel electrophoresis. The data shown represent one of five reproducible results of different experiments.

Reduction of the mRNA level of IFN-γ and IRF-1 by gamma irradiation Confluent splenocytes from Balb/C mice were irradiated with a dose of 5 Gy, then stimulated with Con A at 3 h post-irradiation. After another 3 h of incubation, the total RNA was isolated and RT-PCR was performed. The mRNA of IFN-γ was significantly reduced to 40% of the control level by gamma irradiation (Fig. 2). Interferon regulatory factor (IRF)-1 is a transcription factor that is induced by IFN-γ stimulation (Watowich S.S. *et al*., 1996). The induction of IRF-1 mRNA is controlled by IFN-γ (Pine, R. *et al*., 1994); therefore, we also examined the mRNA expression of IRF-1. IRF-1 was reduced to 50% of the control mRNA expression level 6 h after irradiation (Fig. 3). The reduction of the IRF-1 mRNA level by gamma irradiation appeared as a consequence of the reduction of the IFN-γ

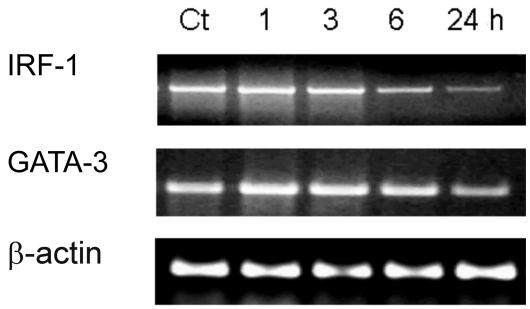

Fig. 3. Alteration of the mRNA expression of transcription factors related with cytokines by gamma irradiation. Murine splenocytes were stimulated with Con A (2.5 μg/ml) for 2 days, followed by gamma irradiation. The total RNA was extracted at the indicated time, and a RT-PCR analysis was performed. One microgram of intact total RNA was reversibly transcribed into first-strand cDNA and amplified using PCR. The products were analyzed on 1% agarose gel electrophoresis. The data shown represent one of two reproducible results of different experiments.

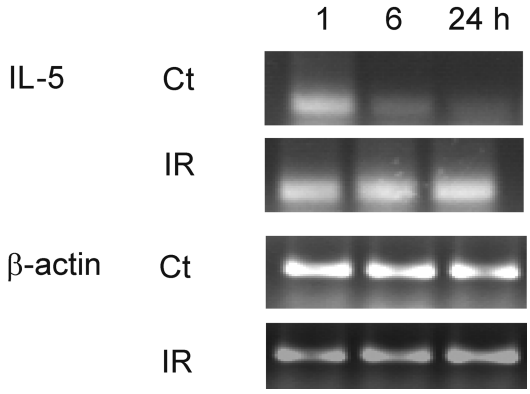

Fig. 4. Modulation of IL-5 levels in Con-A stimulated splenocytes by γ-irradiation. The splenocytes were irradiated by 5 Gy of γ-ray and stimulated with Con A (2.5 μg/ml) 5 min after gamma irradiation. At 1, 6, or 24 h post-irradiation, the total RNA was purified from the irradiated and stimulated cells. One microgram of total intact RNA was reversibly transcribed into first-strand cDNA and amplified using PCR. The PCR products were analyzed on 1% agarose gel electrophoresis. The data shown represent one of five reproducible results of different experiments.

mRNA expression. Therefore, the gamma irradiation probably reduced the mRNA expressions of Th1 cytokine, IFN-γ, and its related transcription factor, IRF-1.

Induction of the mRNA expression of IL-5 and GATA-3 by gamma irradiation Confluent splenocytes from Balb/C mice were irradiated with a dose of 5 Gy, then stimulated with Con A at 5 min post-irradiation. After another 1, 6, and 24 h of incubation, the total RNA was isolated and RT-PCR was performed. The mRNA of IL-5 was increased by γ-irradiation and the induction was maintaned for 24 h. GATA-3, a

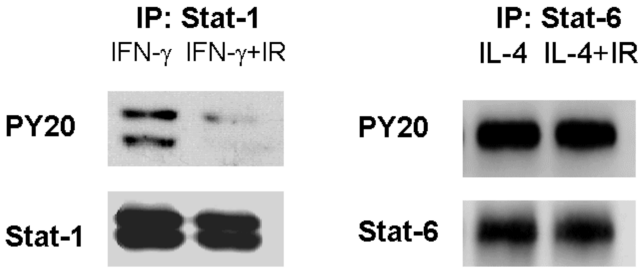

Fig. 5. Effects of gamma irradiation on the phosphorylation of Stat-1 and Stat-6. The splenocytes that were isolated from Balb/c mice were either irradiated or not irradiated, then cultured for 15 min in the presence of IFN-γ (10 ng/ml). Soluble protein that was extracted from each group was immunoprecipitated with anti-Stat-1 and anti-Stat-6 Ab. Western blotting was performed with anti-phosphotyrosine (Abs) (PY20). The amount of Stat was confirmed by blotting with anti-Stat-1 Ab and anti-Stat-6 Abs.

transcription factor that is selectively expressed on naive and Th2 cells, is known to inhibit Th1 development, and modulate IL-4 and IL-5 production (Zhang, *et al*., 1997), as well as to suppress the IFN-γ production (Ouyang, *et al*., 1998). Since GATA-3 not only regulates the IL-4 transcription, but can also activate the transcription of the IL-5 promoter, this led us to also examine the GATA-3 mRNA expression. GATA-3 was also slightly induced in response to gamma irradiation within 1-3 h (Fig. 3). From these results, it appears that gamma irradiation contributes to the induction of the Th2 response by modulating transcription factors that are involved in the Th1 vs. Th2 balance.

Inhibition of the phosphorylation of STAT1 by gamma irradiation In order to address whether or not gamma irradiation interferes with the phosphorylation of STAT1, lysate proteins from Con A-stimulated and irradiated splenocytes were immunoprecipitated with Ab against STAT1. A Western blot analysis was performed with Ab against phosphotyrosine and STAT1. Stimulation of splenocytes with IFN-γ resulted in the phosphorylation of STAT1 without a change in the protein expression of the Stat molecules. Stat is activated in response to cytokines within 5 min, and inactivated rapidly. Therefore, in order to observe the activation of Stat, cytokine must be added, and the signal examined within 15 min. The tyrosine phosphorylation of STAT1 was inhibited by gamma irradiation (Fig. 5). However, the phosphorylation of STAT6, which is involved in the signaling of Th2 cytokine IL-4, was not inhibited by gamma irradiation (Fig. 5). Therefore, the suppressive effect of gamma irradiation on STAT activation was STAT1-specific.

The functional activity of T lymphocytes was reduced by gamma irradiation The generation of cytotoxic T lymphocytes (CTL) by Th1 type cytokines is essential in the host response to tumors, transplants, and viruses. The CTL generation is particularly related with the mixed-lymphocytes

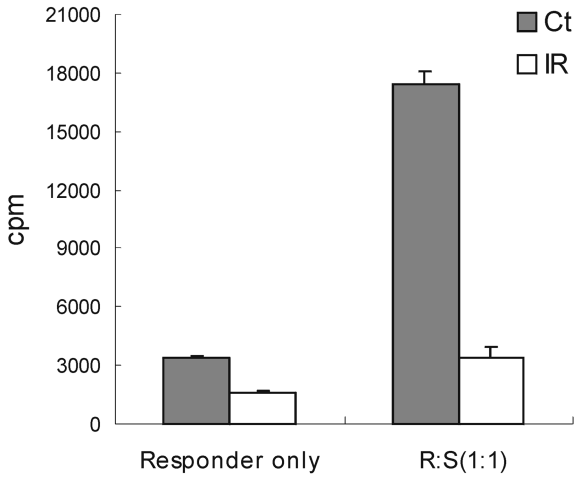

Fig. 6. Effects of gamma irradiation on the proliferating activity of T lymphocytes. Five days after the whole-body irradiation, the mice of each group were sacrificed and the splenocytes were isolated (Ct: control group, IR: irradiated group). The splenocytes of each group were used as responder cells (Balb/c: H-2^d) and cultured in the presence of prepared stimulator cells (C57BL/6: H-2^b), irradiated at the dose of 20 Gy at the ratio of 1 : 1 (responder: stimulator) in a flat-bottomed 96-well microplate for 4 days. The amount of ^{3}[H]-thymidine that was incorporated was measured by a β-counter. This experiment was carried out in triplicate from three different preparations. The values are presented as mean ± SE.

reaction (MLR). To evaluate the effect of IR on the function T-lymphocytes, allogenic MLR was performed. At 5 days after whole-body irradiation, the mice were sacrificed and the splenocytes that were isolated from each group (Balb/c; H-2^d) were used as the responder. These were cultured with the prepared stimulator (C57BL/6; H-2^b) at the ratio of 1 : 1 (responder : stimulator) on a flat-bottomed 96-well microplate for 4 days. The control was cultured in the absence of a stimulator. The amount of ^{3}[H]-thymidine that was incorporated into the proliferating responder was measured by a β-counter. The activity of the irradiated group (IR) was 20~22% of the control group (Ct) (Fig. 6). Thus, it was suggested that the cell-mediated immunity was inhibited by gamma irradiation, which was related to the reduction of Th1 cytokine, IFN-γ.

Discussion

Gamma irradiation not only causes DNA damage, but also triggers a variety of physiologic responses in mammalian cells, including immunosuppressive effects and inflammation. The difference in the radiosensitivity of the cells that are involved in immune responses is well known, yet little is known about the regulation of the cytokine release by irradiated cells. In particular, the immunosuppression mechanism of gamma irradiation is not yet understood. Therefore, we examined the effects of gamma irradiation on the regulation of cytokine release, and investigated the underlying molecular mechanism.

In the present study, gamma irradiation caused a reduction in the IFN-γ gene expression and the relative induction of the IL-5 gene expression in the splenocytes that were activated with Con A. (to amplify the sensitive effects of gamma irradiation). We observed that IFN-γ was reduced 3 h post-irradiation, and IL-5 was induced rapidly after irradiation in naive splenocytes (data not shown). Therefore, Con A was added 3-h post-irradiation for the IFN-γ measurement, and 15-min post-irradiation for IL-5. We also examined another Th1 type and Th2 type cytokine gene expression. We observed the reduction of the Th1 type, such as IL-2, and the induction of Th2 type, such as IL-4 and IL-10 in activated and naïve splenocytes (manuscripts in preparation). Thus, the differential regulation of the cytokine expression by gamma-irradiation was able to alter the balance of the Th1 and Th2 immune responses. Previously, different radiosensitivities among lymphocyte subpopulations were demonstrated. The Th1 cells are more radiosensitive than the Th2 cells (Galdiero, *et al*., 1994). We selected 5 Gy as a sublethal dose to conduct this study, which is based on MTT data. Therefore, the differential mRNA expressions of the Th1 and Th2 type cytokines in this study were not a result of the different radiosensitivities of the Th cell type for cell survival. Throughout this study, the non-irradiated and irradiated cells were exposed to exactly the same conditions (except irradiation). Accordingly, it was evident that the immunosuppression that was caused by gamma irradiation was due to the differential regulation of the Th1 and Th2 type cytokine gene expressions.

To verify the differential regulation of the Th1/Th2 cytokine at the transcription level, we examined the expression levels of the cytokine-mediated transcription factors, such as IRF-1 and GATA-3. We also investigated the activation of the cytokine receptor-mediated signaling molecules, STATs. IFN-γ and IL-12 promoted Th1 differentiation. The production of IFN-γ was directly related to the induction of the transcription factor, IRF-1, through the activation of STAT1 (Lohoff *et al*., 1997; Sato *et al*., 1997; Taki *et al*., 1997; Coccia *et al*., 1999). In this study, not only the reduction of IFN-γ and IRF-1 mRNA, but also the reduction of STAT1 phosphorylation was observed in response to IFN-γ by gamma irradiation. The JAK/STAT pathway became rapidly activated within 1~15 min of the receptor stimulation. This led to the rapid translocation of dimerized Stats (Shuai, *et al*., 1993; Silennoinen, *et al*., 1993). This pathway was also rapidly inactivated. The IFN-γ-induced STAT1 signaling demonstrated that the entire signaling cycle is approximately 4 h (Haspel, *et al*., 1996). Moreover, while the STAT1 activation was no longer than 15 min, the overall cellular protein levels of the STAT1 molecule were unchanged during the activation-inactivation cycle (Haspel *et al*., 1996; Lee *et al*., 1997). Thus, we also examined (within 15 min) STAT1 in response to IFN-γ, and observed the inhibition of STAT1 phosphorylation by gamma irradiation. Therefore, the

inhibition of IFN-γ induced STAT1 phosphorylation can cause the inhibition of the IRF-1 mRNA expression, since activated STAT1 interacts with GAS element of the IRF-1 promoter (Pine, *et al*., 1994). In fact, we observed the inhibition of the IRF-1 mRNA expression 1 h after gamma irradiation. The inhibition of the IFN-γ expression by gamma irradiation was accompanied by a reduction in the IRF-1 expression. Therefore, it was thought that the IRF-1 expression might be affected by the reduction of the IFN-γ expression and the inhibition of STAT1 phosphorylation by gamma irradiation. Thus, the cell-mediated immunity that is induced by Th1 cytokines can be down-regulated. Because all of the STAT proteins bind to receptor phosphotyrosine via Src homology region domains, we examined whether or not gamma irradiation generally interferes with the phosphorylation of STAT proteins. However, the IL-4-induced STAT6 activation was unaffected by gamma irradiation. Additionally, the IL-5 expression, Th2 type cytokine, was rapidly induced in response to gamma irradiation. GATA-3, which is a transcription factor that is selectively expressed on naive and Th2 cells, inhibits Th1 development, and modulates IL-4 and IL-5 production (Zhang, *et al*., 1997), as well as inhibiting IFN-γ production (Ouyang, *et al*., 1998). Therefore, the production of IL-5 could be enhanced by the GATA-3 transcription factor. In this study, gamma irradiation induced the mRNA expressions of GATA-3 (Fig. 3), which could affect the IL-5 expression level. Therefore, the increase of GATA-3 by gamma irradiation could contribute to the differential regulation of the Th1 and Th2 cytokine expressions. According to these results, it was proposed that the modulation of the Th2 type cytokine and related transcription factors by gamma irradiation could potentate the inhibition of the Th1 type cytokine expression. Thus, gamma irradiation was able to inhibit the Th1 immune response through the differential regulation of the cytokine gene expression and interfere with its signaling pathway.

In accordance with the reduction of the Th1 type cytokines by gamma irradiation, it was expected that the generation of cytotoxic T lymphocytes (CTL) would fail in irradiated cells, since Th1 type cytokines (such as IFN-g and IL-2) are very important cytokines in the generation of CTL (Lohoff *et al*., 1997; Kim *et al*., 1999). CTLs are essential in the host response to tumors, transplants, and viruses. The CTL generation is particularly related with MLR, because the stimulator cells also recognize alloantigens on the responder cells (Kim *et al*., 1999). To evaluate the reduction of the cellular immune response by gamma irradiation *in vivo*, allogenic MLR was conducted. Responder cells from Balb/c mice ($H\text{-}2^d$) were stimulated with splenocytes from C57BL/6 mice ($H\text{-}2^b$) that were irradiated at a dose of 20 Gy. The functional activity of the irradiated T lymphocytes was only 20~22% in comparison with that of the control group (Fig. 6). Therefore, the reduction of the Th1 immune response by gamma irradiation was confirmed by this allogenic MLR data. This result may be due to the decrease of the Th1 type cytokine, IFN-γ. Therefore, we propose that gamma radiation caused the reduction of the Th1 cytokine expression and cell-mediated immunity, which could be related to the inhibition of the IFN-γ induced STAT1 activation and related gene activations. Presently, there is no evidence as to whether or not gamma radiation directly inhibits STAT1 phosphorylation. We suggest that the STAT1 protein may be a target of gamma radiation. It could play an important role in the differential regulation of the Th1/Th2 cytokine gene expressions and mediate immunosuppression.

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