Z-100, an Immunomodulatory Arabinomannan Extracted from *Mycobacterium tuberculosis* Strain Aoyama B, Augments Anti-tumor Activities of X-Ray Irradiation against B16 Melanoma in Association with the Improvement of Type 1 T Cell Responses

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In this study, the effects of combination therapy consisting of X-ray irradiation and Z-100 on the survival time of C57BL/6 mice inoculated with B16F10 melanoma were investigated. Survival time was significantly prolonged in B16F10 melanoma-bearing mice treated with the X-ray irradiation (5 Gy) and Z-100 (10 mg/kg s.c.) combination therapy compared with mice irradiated with X-rays alone. The weight of primary tumors and number of metastatic colonies were also significantly suppressed by the combination therapy compared with that in the X-ray irradiation group. These results indicated that Z-100 could enhance the anti-tumor effects of radiotherapy against B16F10 melanoma. On the other hand, the survival time of CD4 knockout mice bearing the same tumors was not prolonged by the combination therapy compared with mice irradiated with X-rays alone, suggesting that CD4⁺ cells are partly involved in augmentation of the anti-tumor effect of radiotherapy by Z-100. In addition, type 1 cytokine (IL-2, IFN-γ) production was significantly increased and type 2 cytokine (IL-4, IL-10) production was significantly suppressed in the tumor-bearing mice treated with the combination therapy compared with the X-ray irradiation group. Moreover, interleukin-12 production by CD11c⁺ cells was also significantly increased in mice treated with the combination therapy compared with the X-ray irradiation group. These results indicate that Z-100 augmented the anti-tumor effects of X-ray irradiation. Moreover, we demonstrated that the effects of Z-100 were expressed at least in part, by the improvement of the T cell responses from type 2-dominant to type 1-dominant via up-regulation of IL-12 production.

**Key words** Z-100; radiotherapy; immunomodulator; T cell response; *Mycobacterium tuberculosis*

The suppressions of primary tumor growth and tumor metastasis are important factors in the therapy of patients with cancer. To date, radiotherapy has been conducted to suppress the growth and metastasis of tumor cells. However, it is difficult to completely damage tumor cells only using radiotherapy, and radiotherapy causes side effects such as impaired immunological function and decreased resistance to opportunistic infections. Based on this knowledge, it is believed to be difficult to cure cancer patients using radiotherapy alone. In order to solve such problems, radiotherapy in combination with immunotherapy is currently being attempted in clinical and pre-clinical studies. That is, the immunotherapy is conducted with the intention of enhancing the anti-tumor effects of radiotherapy via activation of the host immuno-system and alleviation of the side effects caused by the radiotherapy.

Z-100 is polysaccharides composed mainly of arabinomannan, obtained from a hot water extract of *Mycobacterium tuberculosis* strain Aoyama B. In previous reports, Z-100 was shown to have various immunopotentiating activities, including enhancement of the granulopoietic activity of the reticuloendothelial system, a protective effect against *Pseudomonas aeruginosa* infection in immunosuppressed mice, herpes virus infections in thermally injured mice, LP-BM5 murine leukemia virus infection, and induction of cytokines such as IL-1, IL-3, IFN-γ, and IL-12. In Japan, Z-100 is clinically used in patients with leukopenia caused by radiotherapy. It was previously reported that Z-100 prolonged survival time in mice irradiated with a lethal dose of γ-ray (8.5 Gy, whole body) by recovery of hematopoiesis. On the other hand, it was reported that interleukin (IL)-12 and interferon (IFN)−γ, induced by Z-100, lead to an improvement of type 1/2 T cell responses from type 2-dominated states to the normal states, resulting in suppression of tumor metastasis in mice bearing highly metastatic B16F10 melanoma cells (B16F10 melanoma). Furthermore, Z-100 improved the balance of type 1/2 T cell responses in Meth-A tumor cell-bearing mice through up-regulation of IL-12 production from macrophages and IFN-γ production from CD4⁺ cells. Based on these reports, type 1/2 T cell responses are considered to be related to the anti-tumor activities of Z-100 in the tumor-bearing state. The purpose of the present study was to investigate whether Z-100 enhances the anti-tumor activities of radiotherapy in mice bearing B16F10 melanoma. Moreover, we also examined the role of CD4⁺ cells and the improvement of type 1/2 T cell responses on these activities in combination therapy consisting of X-ray irradiation and Z-100.

**MATERIALS AND METHODS**

**Animals** Six- to 7-week-old male C57BL/6 mice were purchased from Charles River Japan. CD4 knockout (KO) C57BL/6 mice (5- to 6-weeks-old, male) were purchased from Taconic, U.S.A. The mice were maintained in aluminum cages with Paper Clean (Japan SLC) bedding under specific pathogen-free conditions in an animal with room controlled temperature and humidity (23 ± 3 °C, 55 ± 20%, respectively). Mice were given CRF-1 feed (Oriental Yeast) and water ad libitum. All animal experiments were approved by the Animal Care and Use Committee of the Central Research Laboratories of Zeria Pharmaceutical Co., Ltd.

**Z-100** Z-100 was produced by Zeria Pharmaceutical Co., Ltd., Tokyo, Japan. C57BL/6 mice or CD4 KO mice

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bearing B16F10 melanoma were subcutaneously administered into the tail base with Z-100 (10 mg/kg) once daily beginning one day after tumor inoculation. As a control, saline was injected according to the same schedule.

**Reagents** RPMI 1640 medium (Nissui Seiyaku), minimum essential medium (MEM) (Invitrogen), Dulbecco’s phosphate-buffered saline (PBS) (Invitrogen), fetal bovine serum (FBS) (JRH Biosciences and Hyclone), IFN-γ, IL-2, IL-4, and IL-10 enzyme linked immunosorbent assay (ELISA) kits (R&D Systems), IL-12 (p40 and p70) ELISA kits (Endogen), anti-CD 3 monoclonal antibody (mAb) (clone 145-2C11, Pharmingen), anti-CD40 mAb (clone 3/23, Pharmingen), mouse CD11c (N418) MicroBeads (Miltenyi Biotec), and a whole-blood erythrocyte lysing kit (R&D Systems) were used in the experiments. Other reagents were extra fine grade.

**Implantation of B16F10 Melanoma in C57BL/6 Mice or CD4 KO Mice** C57BL/6 mice or CD4 KO mice were inoculated in the right footpad with B16F10 melanoma (5×10^6 cells/mouse). The survival times of the mice were observed daily. The lung tissues were removed from B16F10 melanoma-bearing mice 21 d after tumor inoculation. The number of metastatic colonies in the lungs were counted under a dissecting microscope after fixing the lung tissue with 10% formaldehyde solution. To obtain primary tumors, knee joints were excised 21 d after tumor inoculation and the primary tumor weight was measured using an electric balance.

**Local Irradiation of X-Ray** The mice were immobilized and then the entire body of the mice, except for the area of the primary tumors was shielded by lead blocks. The primary tumor sites were locally irradiated with X-rays (5 Gy) using a Sofron NST-1005C.

**Induction and Measurement of Type 1/2 Cytokine Production by CD4+ Cells** Splenocytes (three mice pooled) were prepared from normal mice or B16F10 melanoma-bearing mice 21 d after tumor inoculation. The single-cell suspensions were passed through nylon mesh (Becton Dickinson) and washed with RPMI 1640 medium by centrifugation for 5 min at 1200 rpm. The erythrocytes were removed with a whole-blood erythrocyte lysing kit and then washed twice with medium. To prepare CD4+ cells, splenocytes were passed through a CD4+ T cell subset column (Cytovax Biotechnologies). This procedure resulted in a population of >84% of CD4+ cells, as assayed by flow cytometry. The CD4+ cells were suspended in RPMI 1640 supplemented with 10% heat-inactivated FBS, antibiotics, and L-glutamine (2 mmol/l). The number of viable cells was determined using the trypan blue dye-exclusion method and then 2×10^6 cells/ml of these cells were stimulated with anti-CD3 mAb (2.5 μg/ml) in a 96-well tissue culture plate and incubated for 48 h at 37°C in 5% CO2. The culture fluids harvested were stored at −80°C until use and assayed for the amounts of type 1 cytokine (IL-2, IFN-γ) and type 2 cytokine (IL-4 and IL-10) using ELISA kits.

**IL-12 Production by CD11c+ Cells** The splenic CD11c+ cells (three mice pooled) were prepared from normal mice or B16F10 melanoma-bearing mice 21 d after tumor inoculation. A magnetic cell sorting system (MACS) was used to separate CD11c+ cells from splenocytes. Briefly, splenocytes were mixed with magnetic-beads conjugated anti-mouse CD11c+ (N418) mAb and incubated at 4°C for 15 min. Then the splenocytes were washed and suspended in cell separation buffer. The cell separation columns were set to the magnet for the Midi MACS (Miltenyi Biotec), and the cell suspensions were applied to the cell separation column. After washing the column with cell separation buffer to elute the non-attached cells completely, the attached cells were eluted. The CD11c+ cells were suspended with MEM supplemented with 10% heat-inactivated FBS, antibiotics, L-glutamine (2 mmol/l), and 2-mercaptoethanol (5×10^-2 mol/l). Then 2.5×10^6 cells/ml of these cells were stimulated with anti-CD40 mAb (10 μg/ml) in a 96-well tissue culture plate for 24 h at 37°C in 5% CO2. The culture fluids harvested were stored at −80°C until use and the amount of IL-12 (p40 and p70) assayed using an ELISA kit.

**Statistical Analysis** Data are expressed as the mean±standard error (S.E.). Statistical analysis was performed using the Dunnett and Tukey tests. Statistical differences in survival times were calculated using the Kaplan–Meier method (Log-rank test). *p*-Values less than 0.05 were considered as significant.

**RESULTS**

**Increase in Survival Time of B16F10 Melanoma-Bearing Mice Treated with the Combination Therapy of X-Ray Irradiation and Z-100** The anti-tumor effect of combination therapy consisting of X-ray irradiation and Z-100 was investigated in terms of the survival time of mice inoculated with B16F10 melanoma. C57BL/6 mice were inoculated into the right hind footpad with B16F10 melanoma (5×10^6 cells/mouse). In the Z-100-treatment group and the combination therapy group, the mice were subcutaneously administered Z-100 (10 mg/kg) daily into the tail base from the next day of tumor inoculation. In the tumor control group and the X-ray irradiation group, the same tumor-bearing mice were administered saline according to the same schedule. In the X-ray irradiation group and the combination therapy group, primary tumors were locally irradiated with X-rays (5 Gy) 10 d after tumor inoculation. As shown in Fig. 1, all of the tumor-bearing mice in the tumor control group died within 29 d after tumor inoculation. The survival times of tumor-bearing mice treated with X-ray irradiation alone (*p<0.01) and the combination therapy (*p<0.001) were significantly prolonged compared with that in the tumor control group. The survival time of the Z-100-treatment group was slightly longer than that in the tumor control group (*p=0.14). In addition, the survival time of those mice treated with the combination therapy was significantly prolonged compared with that in the X-ray irradiation group (*p<0.05). These results clearly showed that Z-100 significantly enhanced the prolongation of survival time in tumor-bearing mice treated with radiotherapy alone.

**Suppression of Primary Tumor Growth and Pulmonary Metastasis in B16F10 Melanoma-Bearing Mice Treated with the Combination Therapy of X-Ray Irradiation and Z-100** To determine the effects of combination therapy on the growth of primary tumors and spontaneous metastasis in B16F10 melanoma-bearing mice, the tumor-bearing mice were administered Z-100 (10 mg/kg s.c.) every day beginning 1 d after tumor inoculation. The primary tu-
mors were locally irradiated with 5 Gy of X-rays once on day 10. The primary tumors were weighed and the number of pulmonary metastases was counted 21 d after tumor inoculation. Tumor growth (Fig. 2a) and tumor metastasis (Fig. 2b) were significantly suppressed in the tumor-bearing mice irradiated with X-rays alone (\(p<0.05\)), administered Z-100 alone (\(p<0.05\)), and treated with the combination therapy (\(p<0.05\)) compared with the tumor control group. Furthermore, tumor growth and spontaneous metastasis were significantly suppressed in the tumor-bearing mice treated with the combination therapy compared with the X-ray irradiation group (\(p<0.05\)). The suppression patterns of tumor growth and tumor metastasis paralleled one another.

**Role of CD4^{+} Cells in Anti-tumor Activities of Combination Therapy Consisting of X-Ray Irradiation and Z-100**

CD4 knockout (KO) C57BL/6 mice are deficient in CD4 antigen expression on the cell surface, and are important laboratory animals for examining the function of CD4 antigen-expressing cells. Since CD4^{+} cells have been shown to be immune regulatory cells through the induction of type 1/2 cytokines, the role of CD4^{+} cells in the anti-tumor effects of the combination therapy was investigated using CD4KO mice bearing B16F10 melanoma. CD4KO mice were inoculated into the right hind footpad with B16F10 melanoma (5\(\times\)10^{5} cells/mouse). In the Z-100 treatment group and the combination therapy group, CD4KO mice bearing the same tumors were administered Z-100 (10 mg/kg s.c.) daily into the tail base from the next day of tumor inoculation. In the tumor control group and the X-ray irradiation group, the tumor-bearing CD4KO mice were administered saline. In the X-ray irradiation group and the combination therapy group, the primary tumors were locally irradiated with X-rays (5 Gy) 10 d after tumor inoculation. As shown in Fig. 3, all of the CD4KO mice bearing B16F10 melanoma as a tumor control group died within 31 d after tumor inoculation. The survival time was significantly prolonged in the tumor-bearing CD4KO mice treated with X-ray irradiation alone (\(p<0.001\)) and the combination therapy (\(p<0.001\)) compared with the tumor control group. These results indicated that the anti-tumor activity of X-ray irradiation was unnecessary CD4^{+} cells (host immuno-system) because the X-ray irradiation directly impaired the growth of tumor cells. The prolongation of survival time was not observed in the tumor-bearing CD4KO mice administered Z-100 alone compared with that...
in the tumor control group. Furthermore, the prolongation of survival time was not observed in the CD4KO mice treated with the combination therapy compared with that in the X-ray control group. These results suggested that CD4⁺ cells are needed for augmentation of the anti-tumor effects of radiotherapy by Z-100, at least in part.

Regulatory Effects of Combination Therapy on T Cell Responses in B16F10 Melanoma-Bearing Mice

To determine the regulatory effects of combination therapy on T cell responses in B16F10 melanoma-bearing mice, the tumor-bearing mice were administered Z-100 (10 mg/kg s.c.) every day beginning 1 d after tumor inoculation. The primary tumors were locally irradiated with 5 Gy of X-rays once 10 d after tumor inoculation. The amounts of IL-2 and IFN-γ produced in the culture fluids were determined using ELISA kits. The results are expressed as the mean±S.E. (n=3 experiment). **p<0.01, compared with normal, + + p<0.01, compared with tumor control, # p<0.05, ## p<0.01, compared with X-ray control (Tukey test).

IL-4 and IL-10 production (type 2 T cell responses) by splenic CD4⁺ cells prepared from the tumor-bearing mice was continuously examined (Fig. 5). IL-4 and IL-10 productions were significantly increased in the tumor-bearing mice compared with the normal mice. In addition, IL-4 and IL-10 productions were significantly suppressed in the tumor-bearing mice treated with X-ray irradiation, Z-100 alone, or the combination therapy compared with the tumor control group. Further, IL-4 and IL-10 productions were significantly suppressed in the tumor-bearing mice treated with the combination therapy compared with the X-ray irradiation group. These results indicated that the T cell responses shifted to the type 2-dominant state in mice inoculated with B16F10 melanoma. Also, X-ray irradiation and the administration of Z-100 and the combination therapy to the tumor-bearing mice improved the type 1/2 immune balance from type 2-dominant to type 1-dominant. Furthermore, the combination therapy significantly enhanced the type 1 T cell responses of the X-ray irradiation group.

IL-12 Production by CD11c⁺ Cells Prepared from B16F10 Melanoma-Bearing Mice Treated with the Combination Therapy

It is known that the balance of type 1/2 T cell responses is regulated by IL-12 produced by dendritic cells (CD11c⁺ cells) that are antigen-presenting cells. In order to determine the regulatory effect on the balance of type 1/2 T cell responses by Z-100 via production of IL-12, IL-12 production by splenic CD11c⁺ cells prepared from the tumor-bearing mice was examined (Fig. 6). In the tumor con-
The experimental methods are the same as in Fig. 1. CD11c+ cells (2.5×10^6 cells/ml), prepared from the mice 21 d after tumor inoculation, were incubated in the presence of anti-CD40 mAb (10 μg/ml) for 24 h at 37 °C in 5% CO2. The amounts of IL-12 produced in the culture fluids were determined using ELISA kits. The results are expressed as the mean±S.E. (n=3 experiments). **p<0.01, compared with normal, + + p<0.01, compared with tumor control, ### p<0.01, compared with X-ray control (Tukey test).

trol group, IL-12 production by CD11c+ cells was significantly decreased compared with that in normal mice. On the other hand, IL-12 production was significantly increased in the tumor-bearing mice irradiated with X-rays, administered Z-100, and treated with the combination therapy compared with that in the tumor control group. Also, IL-12 production was significantly increased in the same tumor-bearing mice treated with the combination therapy compared with that in the X-ray irradiation group.

**DISCUSSION**

Radiotherapy for cancer patients is often performed with immunomodulators because it is able to improve hematopoiesis and enhance the anti-tumor activity of irradiation. Various immunomodulators, such as streptococcal preparation OK-432,15) 1→3-β-D-glucan,16,17) heat-killed Lactobacillus casei preparation,18) and alpha-galactosylceramide19) have been reported to enhance the anti-tumor activities of radiotherapy in clinical or pre-clinical trials. These reports indicated that the combination of radiotherapy and immunotherapy could be useful for treating patients with cancer through augmentation of host defense mechanisms by immunotherapy. In this paper, we demonstrated that survival time was significantly prolonged in B16F10 melanoma-bearing C57BL/6 mice treated with the combination therapy compared with the X-ray irradiation alone group. Furthermore, both tumor growth and spontaneous metastasis were also suppressed in the same tumor-bearing mice treated with the combination therapy compared with the X-ray irradiation alone group. These results clearly showed that Z-100 immunotherapy significantly enhanced the anti-tumor effects of X-ray irradiation in the tumor-bearing mice, suggesting that it can be applied to treat cancer patients undergoing radiotherapy.

Type 1 and type 2 T cell response patterns are defined both by cytokine secretion and immune function.20,21) In general, the type 1 T cell response pattern is characterized by IL-2 and IFN-γ production and the up-regulation of cell-mediated responses.22,23) The type 2 T cell response pattern is characterized by IL-4 and IL-10 production and the up-regulation of a variety of antibody responses.24) IFN-γ, which is produced by type 1 T cells, induces type 1 T cell differentiation and suppresses type 2 T cell responses.25) On the other hand, IL-4 and IL-10, which are produced by type 2 T cells, have been shown to regulate the differentiation of type 2 T cells and to inhibit the function of type 1 T cells.26,27) Thus, these reports suggested that the control of immune responses constructed by type 1 and type 2 cytokines is carried out ultimately via cytokines. In pre-clinical studies, the balance of type 1/2 T cell responses has been shown to be critically important in anti-tumor immune responses, such as the inhibition of tumor growth and metastasis, and survival rate.28—29) It has been reported that immunomodulators, such as lipoteichoic-acid-related molecule from OK-432,30) 1→3-β-D-glucan,31,32) CpG-DNA,33) glycyrrhizin from licorice roots,34) and Celosia argentea seed extracts35) can regulate the type 1/2 T cell responses from the type 2-dominant to type 1-dominant. In addition, it has been clinically reported that administration of lentinan, anti-tumor polysaccharides36) and OK-43237) to patients with digestive cancer were able to improve T cell responses from the type 2-dominant state to the normal state in cancer patients. Moreover, it has been reported that co-administration of bacille Calmette-Guérin (BCG) and IFN-α enhanced type 1 T cell responses in patients with bladder cancer,38) and that administration of Mycobacterium vaccae (SRL172) to patients with advanced prostate cancer increased the percentage of IL-2 producing cells and decreased the percentage of IL-4 producing cells in peripheral blood mononuclear cells.39 These reports suggested that immunomodulators (immunotherapy), which can improve immune responses from the type 2-dominant to the type 1-dominant, are useful in the treatment of cancer patients because the anti-tumor effects of immunomodulators are induced by the direct effects of type 1 T cells and type 1 cytokines, or the harmonious effects of antigen-presenting cells, cytotoxic T cells, and NK/NKT cells whose functions have been augmented by type 1-dominance. In this study, we demonstrated that survival time in CD4KO mice bearing B16F10 melanoma by the combination therapy was not significantly prolonged compared with that in the X-ray irradiation alone, even if the combination therapy enhanced the prolongation of survival time in wild type mice. These results indicated that CD4+ cells are partly involved in the anti-tumor effects of X-ray irradiation in combination with Z-100. Although Z-100 suppressed the growth and metastasis of tumor cells in wild type mice, the role of CD4+ cells was still uncertain in the inhibition of tumor growth and metastasis by Z-100. The present results clearly revealed that X-ray irradiation, Z-100, and the combination therapy could improve type 1/2 T cell responses to type 1-dominant from type 2-dominant, as observed in mice inoculated with B16F10 melanoma. It was elucidated that the combination therapy significantly enhanced the type 1 T cell responses of the X-ray irradiation group. These results suggested that Z-100 significantly enhanced the anti-tumor effects of X-ray irradiation in association with its acceleration of T cell responses to type 1-dominant in the X-ray irradiation group, at least in part. On the other hand, the rejection of tumors has been shown to be mediated by CD8+ cells and/or NK/NKT cells.
in addition to the involvement of CD4+ cells in many experimental tumor models. Brunda et al. reported that CD8+ cells play a critical role in the anti-tumor activities of IL-12 against B16 melanoma. In addition, NK cells and NKT cells are important effector cells for the anti-tumor activity of IL-12 and/or Th1 cells. Although we focused on the role of CD4+ cells on the anti-tumor activity of Z-100 in the present study, the next step should be to confirm the involvement of CD8+ T cells and NK/NKT cells on Z-100-induced tumor rejection. From that knowledge, the efficacy of combination therapy was decreased in the depleted function of CD4+ cells, but remained role of CD8+ cells, NK cells and NKT cells using these KO mice. We believe it is necessary to identify effector cells with respect to the anti-tumor activities of combination therapy using various kinds of KO mice. Additionally, flow cytometric detection of intracellular cytokines such as IFN-γ and IL-4 is a powerful means with which to identify effector cells. Recently, CD4+ cells were reported to classified as CD4+CD3+NK1.1+ Th cells, CD4+CD3+NK1.1+ NKT cells and CD4+CD3+CD25+ regulatory T cells in their subpopulation. In this manner, analysis of these effector cells remains to be elucidated using above mentioned method. But, it was seemed that Z-100 might induce cellular immunity, acquired type 1 T cell responses, in association with cooperation of above described cells such as CD4+ cells, CD8+ cells, NK cells, NKT cells, antigen-presenting cells, and other cells.

IL-12 is a heterodimeric cytokine that has been shown to have strong anti-tumor activities, mediated in large part by its ability to induce IFN-γ. IL-12 was produced by antigen-presenting cells such as monocytes, macrophages, dendritic cells, and Langerhans cells. Further, IL-12 is also known to play a critical role in the differentiation of Th0 cells to Th1 cells, and Th2 cells to Th0 cells. These reports indicated that IL-12 produced by these cells is a key cytokine in the regulation of immune balance. We found that X-ray irradiation, Z-100, and the combination therapy significantly increased IL-12 production by CD11c+ cells prepared from mice inoculated with B16F10 melanoma. IL-12 production in the combination therapy group was also significantly increased compared with that in the X-ray irradiation group. On the other hand, we have reported that IL-12 is required for the appearance of the anti-metastatic activity of Z-100 and improvement of the balance of type 1/2 T cell responses. These findings suggested that the anti-tumor effects of combination therapy were expressed by the improvement of the T cell responses from type 2-dominant to type 1-dominant via up-regulation of IL-12 production. As described above, X-ray irradiation alone exhibited anti-tumor activities such as the suppression of tumor growth, metastasis, and increased in survival time because X-ray irradiation directly impaired the growth of B16F10 melanoma cells. On the other hand, Z-100 markedly augmented the anti-tumor activities of radiotherapy alone. These results presumed that Z-100 could synergistically augment the anti-tumor activities of radiotherapy because the anti-tumor mechanism of Z-100 was different to that of radiotherapy, namely, the anti-tumor mechanism of Z-100 was the regulation of host immunosystems such as the improvement of the type 1/2 T cell responses and an increase in IL-12 production. Recently, Nikitina et al. reported that the combination of apoptosis-inducing therapy by gamma irradiation and dendritic cell administration resulted in an anti-tumor effect with a significant increase in T cell responses, while each treatment alone had no effect. Kokhaei et al. reported that apoptotic tumor cell-endocytosed dendritic cells induced type 1 T cell responses. Moreover, Makidono et al. reported that radiation-damaged apoptotic tumor cells are readily phagocytosed by macrophages and that tumor specific antigen is presented as a complex with MHC class II antigen on macrophages to CD4+ T cells, and thus irradiation enhances anti-tumor immunity by the induction of antigen-specific helper T cells. These reports and our data presumed that Z-100 augmented the anti-tumor activities of radiotherapy, since Z-100 might enhance type 1 T cell responses through antigen-presenting cells (dendritic cells, macrophages, etc.) recognized with apoptotic tumors by X-ray irradiation, at least in part.

Taken together, these findings indicate that the anti-tumor effects of combination therapy consisting of X-ray irradiation and Z-100 improved the balance of T cell responses from type 2-dominant to type 1-dominant through the up-regulation of IL-12 production by CD11c+ cells, at least in part. Since these studies were carried out in mice, the clinical relevance of these results remains unknown. However, it is thought that immunotherapy using Z-100, which modulates the cellular immune responses as described above, is useful in the treatment of patients with malignant tumors who undergo radiotherapy.

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