

Repeated Irradiations with γ -rays at a Dose of 0.5 Gy May Exacerbate Asthma

Su-ping FANG¹, Fumitoshi TAGO¹, Takashi TANAKA¹, Noriko SIMURA¹,
Yasuko MUTO², Resuke GOTO² and Shuji KOJIMA^{1*}

Radiation/Asthma/OVA.

We previously showed that 0.5 Gy whole-body γ -ray irradiation with a single or small number of repeated exposures inhibits tumor growth in mice, via elevation of the IFN- γ /IL-4 ratio concomitantly with a decrease in the percentage of B cells. Here we examined whether repeated 0.5 Gy γ -rays irradiation can improve asthma in an OVA-induced asthmatic mouse model. We found that repeated irradiation (10 times) with 0.5 Gy of γ -rays significantly increased total IgE in comparison with the disease-control group. The levels of IL-4 and IL-5 were also significantly higher in the γ -ray-irradiated group, while that of IFN- γ was significantly lower, resulting in a further decrease of the IFN- γ /IL-4 ratio from the normal value. These results indicate that the repeated irradiation with γ -rays may exacerbate asthma, and may have opposite effects on different immune reactions unlike the irradiation with a single or small number of repeated exposures.

INTRODUCTION

It is now well known that the response of the immune system to ionizing radiation depends on the dose and the dose-rate. During the last century, it was initially thought that even very low radiation doses were harmful, and that there was a linear dose-effect relationship (so-called linear no-threshold (LNT) theory). However, epidemiological findings on cancer frequency in areas with high natural radiation background and various animal experiments cast doubt on this theory. Since Luckey¹⁾ first proposed physiological stimulatory effects of radiation (so-called hormesis) in 1982, much evidence in support of the concept has been accumulated. It is now accepted that low-dose whole-body irradiation enhances host immune response, rather than having a direct cell killing action.²⁾

In recent years, low-dose irradiation has been examined for the pre-clinical treatment of some chronic diseases, such as chronic lymphocytic leukemia and low-grade non-Hodgkin's lymphoma, and long-term remissions have been

obtained.³⁾ The efficacy of low-dose whole-body irradiation is believed to stem from three mechanisms, namely immune enhancement, induction of apoptosis, and the intrinsic hypersensitivity to low radiation doses of many cell lines and tumors. Data from animal experiments have shown that low-dose whole-body irradiation enhances the immune response through (1) augmenting the proliferative reactive response of T cells to mitogenic stimulation⁴⁾; (2) altering cytokine release, particularly activation of IFN- γ and IL-2 production⁵⁾; (3) increasing the expression of IL-2 receptors on the T-cell surface⁶⁾; (4) facilitating signal transduction in T lymphocytes⁷⁾; (5) increasing splenic catecholamine content and lowering the serum corticosterone level⁸⁾; (6) eliminating a particularly radiosensitive subset of suppressor T cells.⁹⁾

Our recent studies have shown that 0.5 Gy whole-body γ -ray irradiation with a few frequency inhibits tumor growth in mice.^{10,11)} These anti-tumor effects were mediated by the radiation-induced elevation of the IFN- γ /IL-4 ratio concomitantly with a fall in the percentage of B cells. It is well known that asthma is characterized by the production of large quantities of IgE antibodies by B cells and a decrease of IFN- γ /IL-4 ratio. The immuno-modulatory effects of 0.5 Gy whole-body irradiation might therefore act in opposition to the pathogenetic mechanisms of asthma. We therefore examined the effect of γ -ray irradiation on asthma in an ovalbumin-induced asthmatic mouse model.

*Corresponding author: Phone: +81-4-7121-3691 ext 6443,
Fax: +81-4-7121-3613,
E-mail: kjma@rs.noda.tus.ac.jp

¹Department of Radiation Biosciences, Faculty of Pharmaceutical Sciences, Tokyo University of Science, Noda-shi, Chiba 278-8510, Japan; ²Laboratory of Radiation Biology, Graduate School of Nutritional and Environmental Sciences, University of Shizuoka, 52-1 Yada, Shizuoka-shi 422-8526, Japan.

MATERIALS AND METHODS

Animals

Five-week-old male BALB/c mice, weighing 14–16 g, were purchased from Sankyo Laboservice Co. (Tokyo, Japan). They were housed in plastic cages with sterilized wood chips bedding and bred in rooms kept at a temperature of $23 \pm 2^\circ\text{C}$ and a relative humidity of $55 \pm 10\%$ under a 12 h light-dark cycle. They were allowed free access to tap water and experimental normal diet (CE-2, CLEA Co., Ltd., Tokyo, Japan). The mice were separated into 3 groups, i.e., normal, disease-control and irradiation groups. Animals were treated and/or handled according to the Recommendations for Handling of Laboratory Animals for Biomedical Research, compiled by the Committee on Safety and Ethical Handling Regulations for Laboratory Animal Experiments, Tokyo University of Science. All experimental procedures mentioned below were in accordance with institutional guidelines for the care and use of laboratory animals in research.

Materials

Ovalbumin (OVA) was used as an antigen. $\text{Al}(\text{OH})_3$ (13 mg/ml) was purchased from Sigma Chemical Co.. The following antibodies were used in this study: anti-mouse IFN- γ mAb, biotin-conjugated anti-mouse IFN- γ mAb, anti-mouse IL-4 mAb, biotin-conjugated anti-mouse IL-4 mAb, anti-mouse IL-5 mAb, and biotin-conjugated anti-mouse IL-5 mAb (eBioscience, San Diego, CA: XMG1.2, R4-6A2, 11B11, BVD6-24G2, TRFK 5 and TRFK 4, respectively). Recombinant mice IFN- γ , IL-4 and IL-5 were purchased from eBioscience (14-8311-63, 14-8041-62 and 14-7052-81, respectively). The following mAbs were labeled with FITC, PE and Cy-Chrome and directed against the following markers: CD3, T cell receptor associated complex present on all mature T cells; CD4, protein on helper T cells; CD8, protein on cytotoxic T cells; CD19, protein on B cells and Pan-NK, protein on NK cells. These mAbs were purchased from Becton Dickinson Co.. All reagent used in this study were reagent grade.

Induction of asthma model

Mice were sensitized and challenged with OVA according to the method described by Ikeda *et al.*¹²⁾ with a slight modification. Briefly, the disease-control and irradiation groups were sensitized i.p. with 100 μl of OVA solution (0.5 mg/ml in saline) emulsified in 150 μl of $\text{Al}(\text{OH})_3$ on day 3 after the start of the experiment at a dosage of 250 μl . A second sensitization was given 5 days after the initial sensitization. Then, on the following 15, 19 and 23 days, mice were kept in a glass barrel for 30 min and challenged with inhalation of aerosolized OVA solution (10 mg/ml in saline) as an antigen challenge for excitation. The aerosol was generated with

an Omron device (NE-U22, Omron Co.). The normal group mice received mock sensitization with i.p. saline- $\text{Al}(\text{OH})_3$ emulsion and were challenged with an aerosol of saline without OVA.

γ -Ray irradiation

Mice were placed in an apertured lucite cabinet and given whole-body irradiation with γ -rays from a ^{137}Cs source (Gammacell 40, Nordin International, Inc., Canada) at a dose of 0.5 Gy (0.98 Gy/min) each time without anesthesia. The schedule for sensitization, inhalation and irradiation is shown in Fig. 1.

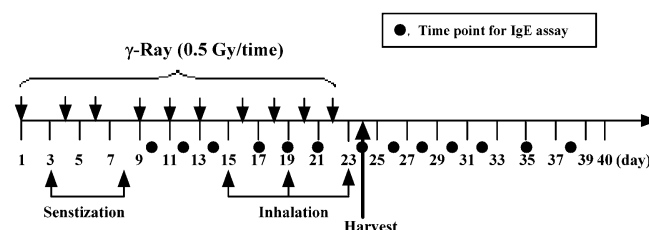


Fig. 1. Schedule for sensitization with OVA emulsified in $\text{Al}(\text{OH})_3$, challenge with aerosolized inhalation of OVA solution and irradiation.

Assay of total IgE

According to the procedure described by Watanabe *et al.*,¹³⁾ blood was collected from the retro-orbital plexus with glass capillary tubes after a full period of irradiation treatment and the plasma was separated. To determine the time-dependent changes in total IgE, blood samples were collected every 2 days from day 5 to day 40 after the start of the experiment. The effect of 0.5 Gy γ -ray irradiation on the IgE level in the OVA-induced asthma model was examined on day 24. Total IgE was measured with an ELISA kit (Bethyl Laboratories, Inc.). The assay sensitivity was 3.9–250 ng/ml, and the samples were appropriately diluted.

Measurement of cytokine production by spleen lymphocytes

Levels of IL-4, IL-5 and IFN- γ produced by spleen lymphocytes were measured as described by Wang *et al.*¹⁴⁾ Briefly, the spleen cells (6×10^6 cells/ml) were harvested on day 24 from each group, suspended in 10% FBS/RPMI 1640 culture and then cultured in the presence of OVA (1 mg/ml) for 72 h at 37°C in the CO_2 incubator. The levels of IL-4, IL-5 and IFN- γ in the culture supernatant were measured with ELISA kits (Pharmingen). The assay sensitivities were 15.625–2000 pg/ml (IL-4 system), 7.8–1000 ng/ml (IL-5 system) and 78.125–5000 pg/ml (IFN- γ system), respectively.

Flow-cytometric analysis of phenotypes of spleen lymphocytes

Spleen lymphocytes were stained with PE-labeled anti-

CD3, Cy-chrome-labeled anti-CD4, FITC-labeled anti-CD8, FITC-labeled anti-CD19 and PE-labeled anti-PanNK. All the antibodies used above were purchased from Pharmingen Co.. Data were acquired using a FACS-Calibur flow cytometer (Becton Dickinson, San Jose, CA) and analyzed with FlowJo™ software (Treestar, Inc.)

Statistical analysis

Values for all measurements were expressed as means $\pm$ S.D. Groups were compared by the use of Levene test, 1-way ANOVA and Scheffe test for comparison between two groups. *P* values of less than 0.05 were considered significant.

RESULTS

Total IgE

Time-dependent changes in the total IgE levels are illustrated in Fig. 2. The total IgE content in OVA-induced asthmatic mice significantly increased from 20 days after the start of the experiment, reaching a maximum level at around 30 days. Thereafter, the level gradually decreased up to 38 days. The average mean values ($n = 5$) of the normal group were 255 ng/ml on day 8, 257 ng/ml on day 14, 205 ng/ml on day 18 and 131 ng/ml on day 24. As shown in Fig. 3, the IgE content of the disease-control group on day 24 was significantly higher (7773 ± 2000 ng/ml, $n = 5$) than that of the non-sensitized normal group, (131.4 ± 53.1 ng/ml, $n = 5$), while the value of the irradiated group (14132 ± 3118 ng/ml, $n = 5$) was higher still.

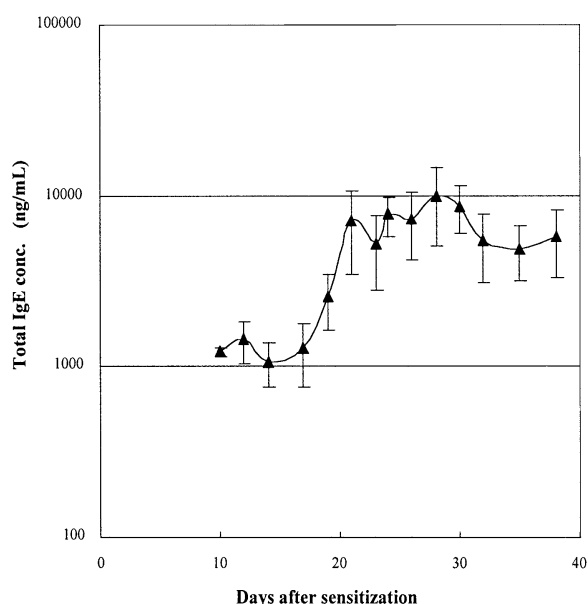


Fig. 2. Time-dependent changes of total IgE level in OVA-induced asthma model mice. Total IgE in plasma post-final inhalation was assayed every two or three days from the 10th to 38th day. The values represent means $\pm$ S.D. of 5 animals.

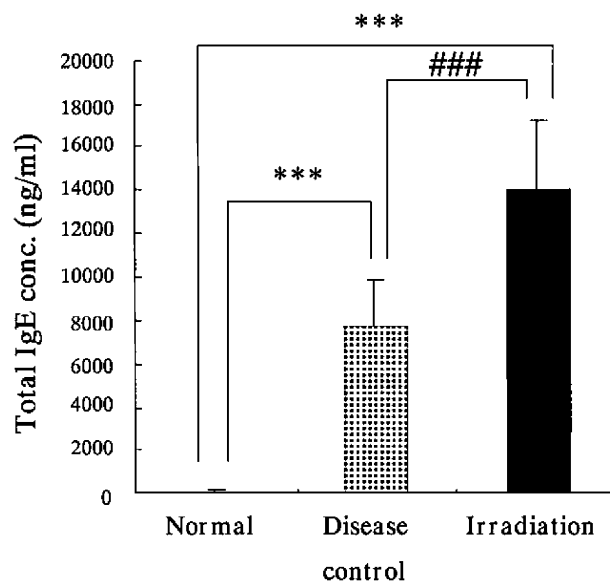


Fig. 3. Effects of 10 repeats of 0.5 Gy γ -ray irradiation on total IgE level on day 24 in OVA-induced asthma model mice. Blood samples were obtained on day 24 after the start of experiment as shown in Fig. 1. The values represent means $\pm$ S.D. of 5 animals. Significant differences from the normal group are indicated with *** ($P < 0.001$), and those from the disease control group with ### ($P < 0.001$).

Effect of 10 repeats of γ -rays on cytokine levels and Th1/Th2 ratio in the OVA asthma model

The production of IL-4 and IL-5 in the disease-control group was significantly increased in comparison with the non-sensitized normal group. These cytokine levels in the irradiated group were both significantly higher than those of the disease-control group (Fig. 4: [A] and [B]). The level of IFN- γ of the disease-control group was also increased significantly compared with the non-sensitized group (Fig. 4: [C]). γ -Ray irradiation clearly lowered the elevated level induced by OVA alone. The ratio of IFN- γ /IL-4 in the disease-control group was reduced to 238.54 ± 24.09 ($n = 5$) compared with 273.41 ± 25.75 ($n = 5$) in the non-sensitized normal group. The IFN- γ /IL-4 ratio of the irradiated group was drastically lowered to 145.37 ± 30.08 ($n = 5$) (Fig. 4: [D]), suggesting exacerbation of asthma. Since IFN- γ and IL-4 are mainly produced by Th1 and Th2 cells, respectively, these results may mean that irradiation inhibits Th1 polarization, but induces Th2 polarization in this asthma disease model.

Effects of 10 repeats of 0.5 Gy γ -ray irradiation on percentage changes of lymphocyte subpopulations in asthma disease model mice

To look at the effect of 10 repeats of 0.5 Gy irradiation on T cell differentiation and inducement from an alternative point of view, we examined the influence of absolute numbers of splenic lymphocytes and percentage changes of lym-

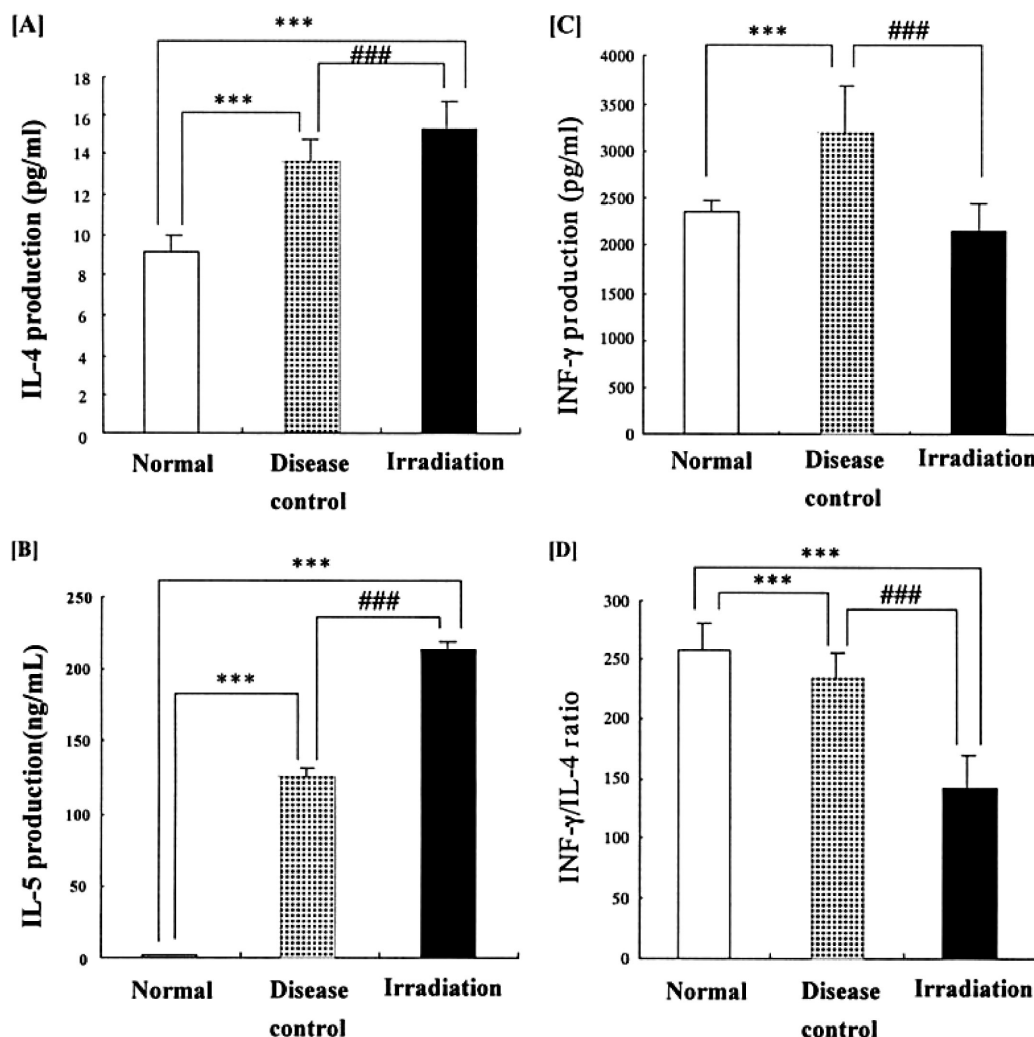


Fig. 4. Effects of 10 repeats of 0.5 Gy γ -ray irradiation on the levels of IL-4, IL-5, IFN- γ and IFN- γ /IL-4 ratio on day 24 in OVA-induced asthma model mice. Panel [A], IL-4; Panel [B], IL-5; Panel [C], IFN- γ ; Panel [D], IFN- γ /IL-4 ratio. Spleen cells were isolated from mice of each group 24 h after the final inhalation on day 23. The cells were cultured in 10% FBS/RPMI 1640 medium in the presence of OVA (1 mg/ml) for 72 h at 37°C in a CO₂ incubator. Each value represents the mean $\pm$ S.D. of 5 animals. Significant differences from the normal group are indicated with *** ($P < 0.001$), and those from the disease control group with ### ($P < 0.001$).

phocyte subpopulations in asthma disease model mice induced by OVA at 24 days after sensitization. The absolute numbers of total lymphocytes in normal, diseases-control, and irradiated group were 1.24×10^7 , 1.26×10^7 , and 0.95×10^7 cells/spleen, respectively. The number of OVA-sensitized/irradiated group was reduced by about 20% in comparison with those of normal and OVA-sensitized groups. As shown in Fig. 5, the percentages of CD3+, CD4+ and CD8+ T cells were significantly decreased after treatment with OVA as compared with the non-sensitized group, while the percentages of CD19+ and Pan-NK were increased compared with the normal group. In contrast, the decreases in the percentages of CD3+ and CD4+ T cells were blocked by the irradiation with γ -rays. The percentages of CD8+ T cells and

Pan-NK in the irradiated group were almost the same as those in the disease-control group, and the percentage of CD19+ was decreased significantly in comparison with that in the disease-control group. These results indicate that the irradiation may activate T cells polarization to helper cells (CD3+CD4+).

DISCUSSION

Early studies on the immunomodulatory effects of low-dose irradiation showed that the reactions of spleen cells to specific antigen (PFC reaction), allotype antigen (MLC reaction) and mitogenic stimulation (LPS reaction and Con A reaction) are enhanced, the activities of NK cells, peritoneal

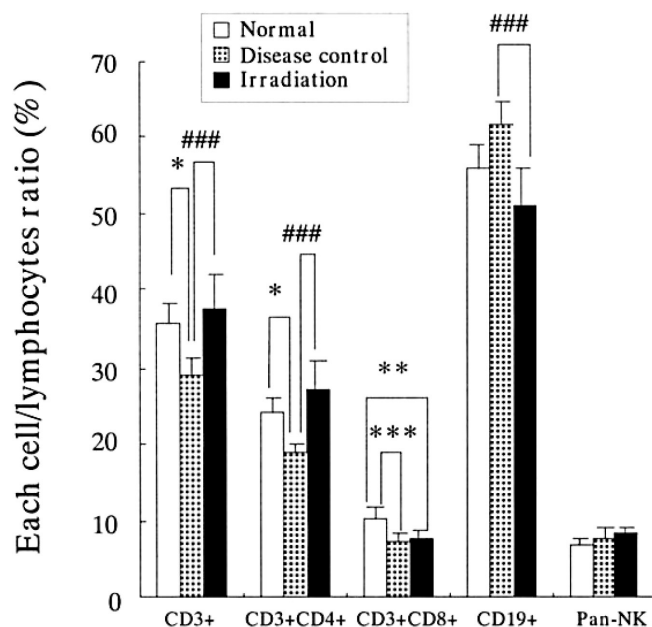


Fig. 5. Effects of 10 repeats of 0.5 Gy γ -ray irradiation on the percentage changes of lymphocyte subpopulations, CD3⁺, CD3⁺CD4⁺, CD3⁺CD8⁺, CD19⁺ and Pan-NK cells, in spleen of OVA-induced asthma model mice on day 24. Lymphocytes were isolated from mice of each group 24 h after the final inhalation on day 23. The absolute numbers of total lymphocytes in normal, diseases-control, and irradiated group were 1.24×10^7 , 1.26×10^7 , and 0.95×10^7 cells/spleen, respectively. Immunophenotyping was carried out by using flow cytometry. Each value represents the means $\pm$ S.D. of 5 animals. Significant differences from the normal group are indicated with * ($P < 0.05$), ** ($P < 0.01$) or *** ($P < 0.001$), and those from the disease control group with ### ($P < 0.001$).

macrophages and ADCC are increased, production of cytokines (such as IL-2 and IFN- γ) by splenocytes is increased, and the differentiation, maturation, renovation and multiplication of immune cells are augmented.^{15,16} Later research on cytokines indicated that after low-dose irradiation, TCR/CD3 on the surface of lymphocytes and IL-2Ra are up-regulated, while the secretion of IL-2, IL-6 and IFN- γ is increased. Therefore, the activation, differentiation and multiplication of T helper cells (TH) play central roles in the immune enhancement.^{17,18} Type 1 cytokines such as IL-2, IFN- γ , and IL-12 are involved in T helper 1 (Th1) immune responses and induce differentiation and cell-mediated immunity, including activation of natural killer cells (NK), cytotoxic T lymphocytes (CTL) and macrophages. By contrast, type 2 cytokines such as IL-4, IL-5, IL-6 and IL-10 are involved in T helper 2 (Th2) immune responses and promote humoral immunity. It is believed that Th1 cytokines such as IFN- γ inhibit Th2 differentiation and Th2 cytokines such as IL-4 inhibit Th1 differentiation.

We previously found that 0.5 Gy γ -ray irradiation of mice elevated the IFN- γ /IL-4 ratio produced by spleen cells and enhanced NK and CTL activity, while decreasing the per-

centage of B cells and increasing the percentage of CD4⁺ T cells in blood at 48 h post-irradiation. The ratio of IFN- γ /IL-4 increased concomitantly with a decrease in the percentage of B cells.^{10,11} Since asthma is characterized by the production of large quantities of IgE antibodies by B cells and a decrease of IFN- γ /IL-4 ratio, we anticipated that 0.5 Gy γ -ray irradiation might improve the status of asthma. Initially, we administered irradiation to the asthma model mice just 2 days before sensitization and OVA inhalation was repeated 5 times, but no effect was observed. When we changed the schedule of irradiation to a total 10 times, however, the levels of total IgE, IL-4 and IL-5 were increased to levels above those of the asthma disease-control group, as shown in Fig. 3 and 4, and the IFN- γ /IL-4 ratio was further decreased. It is noteworthy that irradiation inhibits Th1 polarization, but induces Th2 polarization in this asthma disease model. This result would absolutely be supported by previous reports.^{19–22}

We consider that there might be three possible reasons for these results. Firstly, the total dose of 5 Gy of radiation to the mice was quite high (compared with less than 0.2 Gy for low LET (linear energy transfer) or less than 0.05 Gy for high LET).²³ Secondly, the sensitivity to irradiation may be strain-dependent. Shankar *et al.* indicated that the modification of immune response by radiation was different between BALB/c and C57BL/6 mice (specifically, DTH to *M. vaccae*, augmentation of spleen cells in response to ConA and expression of p53 were different between these two strains).²⁴ Thirdly, we conjecture that irradiation might have an immunostimulatory effect on diseases involving impaired immunity, such as cancer and severe autoimmune disease (e.g., MRL-lpr/lpr mice),²⁵ while exacerbating the response in immune hypersensitivity diseases, such as asthma.

Clearly, further work is needed to establish the effects of whole-body irradiation in various chronic diseases.

REFERENCES

1. Luckey, T. D. (1982) Physiological benefits from low levels of ionizing radiation. *Health Phys.* **43**: 771–789.
2. Liu, S. Z., Bai, O., Chen, D. and Ye, F. (2000) Genes and protein molecules involved in the cellular activation induced by low dose radiation. *Radia. Res. Radiat. Proc.* **18**: 175–186.
3. Safwat, A. (2000) The role of low-dose total body irradiation in treatment of non-Hodgkin's lymphoma: a new look at an old method. *Radiother. Oncol.* **56**: 1–8.
4. Kojima, S., Matsumori, S., Ishida, H., and Yamaoka, K. (2000) Possible role of elevation of glutathione in the acquisition of enhanced proliferation of mouse splenocytes exposed to small-dose γ -rays. *Int. J. Radiat. Biol.* **76**: 1641–1647.
5. Khaskhely, N. M., Maruno, M., Takamiyagi, A., Uezato, H., Kasem, K. M., Hosokawa, A., Kariya, K., Hashiguchi, Y., Landires, E. A., Nonaka, S. (2001) Pre-exposure with low-dose UVA suppresses lesion development and enhances Th1 response in BALB/c mice infected with *Leishmania amazonensis*. *J. Dermatol. Sci.* **26**: 217–232.

6. Xu, Y., Greenstock, C. L., Trivedi, A., Mitchel, R. E. (1996) Occupational levels of radiation exposure induce surface expression of interleukin-2 receptors in stimulated human peripheral blood lymphocytes. *Radiat. Environ. Biophys.* **35**: 89–93.
7. Mori, M., Benotmane, M. A., Vanhove, D., Van, H. P., Hooghe-Peters, E. L., Desaintes, C. (2004) Effect of ionizing radiation on gene expression in CD4+ T lymphocytes and in Jurkat cells: unraveling novel pathways in radiation response. *Cell. Mol. Life. Sci.* **61**: 1955–1964.
8. Liu, S. Z., Han, Z. B., Liu, W. H. (1994) Changes in lymphocyte reactivity to modulatory factors following low dose ionizing radiation. *Biomed. Environ. Sci.* **7**: 130–135.
9. Safwat, A. (2000) The immunobiology of low-dose total-body irradiation: more questions than answers. *Radiat. Res.* **153**: 599–604.
10. Kojima, S., Ishida, H., Takahashi, M., and Yamaoka, K. (2002) Elevation of glutathione induced by low-dose gamma rays and its involvement in increased natural killer activity. *Radiat. Res.* **157**: 275–280.
11. Kojima, S., Nakayama, K., and Ishida, H. (2004) Low dose γ -rays activate immune functions via induction of glutathione and delay tumor growth. *J. Radiat. Res.* **45**: 33–39.
12. Ikeda, Y., Kaneko, A., Yamamoto, M., Ishige, A. and Sasaki, H. (2002) Possible involvement of suppression of Th2 differentiation in the anti-allergic effect of *Sho-seiryu-to* in mice. *Jpn. J. Pharmacol.* **90**: 328–336.
13. Watanabe, M., Asakura, K., Saito, H. and Kataura, A. (1996) Studies on murine of nasal allergy. *Jpn. J. Allergol.* **45**: 1127–1132.
14. Wang, Y., Huang, D. S., Giger, P. T. and Watson, R. R. (1994) Influence of chronic dietary ethanol on cytokine production by murine splenocytes and thymocytes. *Alcohol Clin. Exp. Res.* **18**: 64–70.
15. Liu, S. Z., Liu, W. H. and Qi, J. (1992) The cell mechanisms of immune enhancement by low dose radiation. *Chin. J. Radiol. Med. Prot.* **12**: 299–305.
16. Xu, G. Z., Li, X., Gong, S. L., *et al.* (1995) Effects of radiation on functions of peritoneal macrophages in mice. *J. N. Bethune. Univ. Med. Sci.* **21**: 331–332.
17. Liu, S. Z., Zhang Y. C. and Su, X. (1995) Effect of low dose radiation on the expression of TCR/CD3 and CD25 molecules in the plasma membrane of mouse thymocytes. *Chin. J. Pathophysiology.* **11**: 1–5.
18. Bai, O., Liu S. Z. and Mou, Y. (1998) Effect of low dose radiation on Th1 and Th2 of thymocytes and splenocytes in mice. *Chin. J. Radiol. Med. Prot.* **18**: 106–109.
19. Bass, H., Mosmann, T. and Strober, S. (1989) Evidence for mouse Th1- and Th2-like helper T cells *in vivo*. Selective reduction of Th1-like cells after total lymphoid irradiation. *J. Exp. Med.* **170**(5): 1495–1511.
20. Buttner, C., Skupin, A., Reimann, T., Rieber, E. P., Unteregger, G., Geyer, P. and Frank, K.H. (1997) Local production of interleukin-4 during radiation-induced pneumonitis and pulmonary fibrosis in rats: macrophages as a prominent source of interleukin-4. *Am. J. Respir. Cell Mol. Biol.* **17**(3): 315–325.
21. Haveman, J., Geerdink, A. G. and Rodermond, H. M. (1998) NF, IL-1 and IL-6 in circulating blood after total-body and localized irradiation in rats. *Oncol. Rep.* **5**(3): 679–683.
22. Westermann, W., Schobl, R., Rieber, E. P. and Frank, K. -H. (1999) Th2 cells as effectors in postirradiation pulmonary damage preceding fibrosis in the rat. *Int. J. Radiat. Biol.* **75**: 629–638.
23. UNSEAR (1986) Genetic and somatic effects of ionizing radiation. *Radiat. Prot. Commun.* **1988**, 224
24. Shankar, B., Premachandran, S., Bharambe, S. D., Sundaresan, P. and Sainis, K. B. (1999) Modification of immune response by low dose ionizing radiation: role of apoptosis. *Immunol. Lett. Jun.* **68**: 237–245.
25. Ina, Y. and Sakai, K. (2004) Prolongation of life span associated with immunological modification by chronic low-dose-rate irradiation in MRL-*lpr/lpr* mice. *Radiat. Res.* **161**: 168–173.

Received on November 17, 2004

1st Revision received on February 2, 2005

Accepted on February 2, 2005