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Further Study of Prolongation of Life Span Associated with Immunological Modification by Chronic Low-Dose-Rate Irradiation in MRL-*lpr/lpr* Mice: Effects of Whole-Life Irradiation

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MRL-*lpr/lpr* mice carry a deletion in the apoptosis-regulating *Fas* gene that markedly shortens life due to multiple severe diseases. In our previous study (*Radiat. Res.* 161, 168–173, 2004), chronic low-dose-rate γ irradiation of mice at 0.35 or 1.2 mGy/h for 5 weeks markedly prolonged the life span, accompanied by immunological activation. This report shows that extension of the irradiation period to the entire life of the mice at the same dose rates improved survival further. The 50% survival time for untreated mice, 134 days, was prolonged to 502 days by 1.2 mGy/h life-long irradiation. Also obtained were a time course and a radiation dose-rate response for the activation of the immune system as indicated by a significant increase in $CD4^+$ $CD8^+$ T cells in the thymus and $CD8^+$ T cells in the spleen and also by a significant decrease in $CD3^+$ $CD45R/B220^+$ cells and $CD45R/B220^+$ $CD40^+$ cells in the spleen. Drastic ameliorations of multiple severe diseases, i.e. total-body lymphadenopathy, splenomegaly and serious autoimmune diseases including proteinuria, and kidney and brain-central nervous system syndromes, were found in parallel with these immunological activations, with lifelong low-dose-rate irradiation being more effective than 5-week irradiation at low dose rates. © 2005 by Radiation Research Society

INTRODUCTION

MRL-*lpr/lpr* mice, which carry a mutation of the apoptosis-regulating *Fas* gene, develop multiple severe diseases and have a very short life span, thus providing interesting animal models for experimental therapy (*1–7*). In our previous study, we demonstrated a marked prolongation of the life span of MRL-*lpr/lpr* mice by 5-week γ irradiation at a low dose rate of 0.35 mGy/h and more effectively at 1.2 mGy/h (*8*). These life-span prolongations were associated with immunological activation and amelioration of autoimmune diseases. The present study shows a greater effect of chronic low-dose-rate irradiation continued for almost the entire life span of the mice, together with the results of observations completed for the entire life span of the mice after a 5-week irradiation at low dose rates.

Low-dose radiation induces various effects on living organisms, including augmentation of immunological functions (*9–17*), prevention and cure of diseases (*18–22*), and prolongation of life span (*23–25*). Low-dose radiation activates immune activity in wild-type and *lpr* mice as measured by proliferation of spleen cells to mitogens (*11, 13*) and modifies the latency for radiation-induced myeloid leukemia in CBA/H mice (*26*). Furthermore, low-dose radiation increases the latency of spontaneous lymphomas and spinal osteosarcomas in cancer-prone, radiation-sensitive *Trp53* heterozygous mice (*27*). Recently, Ootsuyama *et al.* (*28*) reported that MRL-*gld/gld* mice, which carry a mutation within the Fas ligand (FasL) and autoimmune diseases similar to *lpr* mice (*29–35*), showed remission of the diseases after low-dose irradiation, in parallel with a decrease in the population of splenic $CD4^-$ $CD8^-$ T cells. They attributed this effect to augmentation of apoptosis.

We show in this report that both the amelioration of multiple severe diseases throughout the body and the prolongation of life span by chronic low-dose-rate irradiation involve the activation of the immune system.

MATERIALS AND METHODS

Materials and methods used were essentially as described previously (*8*).

Animals

Female MRL/MpJUmmCrj-*lpr/lpr* mice (5 weeks old) were purchased from Charles River Japan, Inc., Yokohama, and were kept under specific-pathogen-free conditions. All the animals were maintained on a light schedule from 7:00 to 19:00 and were fed a standard mouse diet CE-2 (Clea Japan, Inc., Tokyo) with water allowed *ad libitum.* The study was reviewed by the Institutional Animal Care and Use Committee, and the mice were treated in accordance with governmental guidelines and the guidelines of the Central Research Institute of Electric Power Industry (CRIEPI).

The urine was tested weekly using commercial test strips (Wako Pure

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Chemical Industries, Ltd., Osaka). Specimens of organs and tissues were fixed in 10% formalin/PBS solution. After embedding in paraffin, 3-μm-thick sections were prepared, stained with hematoxylin and eosin (H&E), and examined histologically under a microscope.

Irradiation

Mice were irradiated in a clean irradiation room equipped with a 370 GBq ^{137}Cs γ-ray source (Chiyoda Technol Co., Tokyo). The dose rates were 1.2 and 0.35 mGy/h, respectively, at a distance of 5 and 10 m from the source as measured by an ionization chamber and glass dosimeter in 2000. The tissue dose rates measured with a glass dosimeter embedded in a mouse's abdomen were 0.95 and 0.30 mGy/h at each point as measured in a separate experiment (*36*). The mice were irradiated continuously except for 1 h in the morning on weekdays. Group a mice (n = 20) were unirradiated and were treated in the same way as the unirradiated controls (n = 12) in the previous experiment (*8*). Group b mice (n = 12) were irradiated with 0.35 mGy/h for 5 weeks, which is the same treatment used in the previous study (*8*), but were observed for the entire life span. Group c mice (n = 20) were irradiated at 0.35 mGy/h for the whole life span. Group d mice (n = 12) were irradiated with 1.2 mGy/h for 5 weeks, which is the same treatment used in the previous study (*8*), but were observed for the entire life span. Group e mice (n = 20) were irradiated at 1.2 Gy for 521 days. The ages of the mice at the start of continuous irradiation were 5 weeks for Groups c and e, and 7 weeks for Groups b and d.

Analysis of Immune Cell Status

Single cell suspensions were prepared from the thymus and spleen in RPMI 1640 medium supplemented with 10% FCS. Immunological changes were examined in terms of cell surface molecules after analysis of cell populations. The cell surface functional molecules CD3, CD4, CD8, CD45R/B220 (B220) and Fas and activation-marker molecule CD40 were analyzed by flow cytometry.

For immunofluorescence studies, the following monoclonal antibodies (mAbs) from BD PharMingen (San Diego, CA) were used: purified 2.4G2 mAb (rat IgG2b, κ), which recognizes CD16/32 (Fcγ III/II receptor) for FcBlock; fluorescein isothiocyanate (FITC)-conjugated 17A2 mAb (rat IgG2b, κ), which recognizes CD3 molecular complex; FITC-conjugated GK1.5 mAb (rat IgG2b, κ), CD4; R-phycoerythrin (R-PE)-conjugated 53-6.7 mAb (rat IgG2a, κ), CD8a (Ly-2); FITC-conjugated and R-PE-conjugated RA3-6B2 mAbs (rat IgG2a, κ), CD45R/B220 (B220); R-PE-conjugated Jo2 mAb (hamster IgG, group 2, λ), Fas (CD95); R-PE-conjugated 3/23 mAb (rat IgG2a, κ), CD40.

The single cell suspensions of thymus and spleen were preincubated with unlabeled anti-Fcγ receptor mAbs for 10 min at 4°C to avoid nonspecific Fc-mediated binding of the labeled antibodies. The cells were stained with FITC-conjugated mAbs and R-PE-conjugated mAbs simultaneously for 20 min at 4°C. After they were washed, the stained samples were analyzed using an EPICS XL flow cytometry system (Beckman Coulter, Inc., Fullerton, CA).

Statistical Analysis

The Kaplan-Meier method was used to estimate the survival curves and the significance of differences between the percentages of survival was evaluated by the logrank test. The significance of differences in the percentages of mice with lymphadenopathy or proteinuria was evaluated by the χ^2 test. The weights of spleen and inguinal lymph nodes and the percentages of cell populations are presented as means ± standard errors of the mean. The statistical significance of the differences was evaluated by the Student's t test.

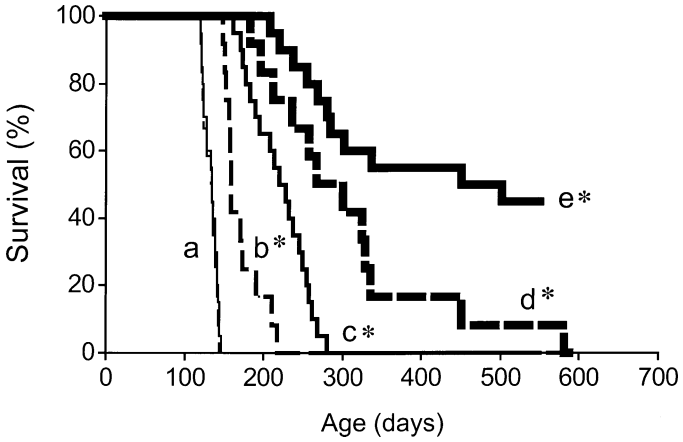

FIG. 1. Prolongation of life span in MRL-*lpr/lpr* mice with chronic low-dose-rate γ radiation. a: Nonirradiated controls. The survival results obtained in our previous observation (*8*) were combined. b: Mice irradiated with 0.35 mGy/h for 5 weeks. The observations in our previous report (*8*) were extended to the entire life span. c: Mice irradiated with 0.35 mGy/h for the whole life span. d: Mice irradiated with 1.2 mGy/h for 5 weeks. Observation until day 203 (*8*) was extended to the entire life span. e: Mice irradiated with 1.2 mGy/h for 521 days. *$P < 0.0001$ compared to controls. $P < 0.0001$ for b − c, d − e, b − d, c − e and b − e.

RESULTS

Chronic Low-Dose-Rate γ Irradiation Prolonged Life Span

MRL-*lpr/lpr* mice were continuously γ-irradiated at a low dose rate of 0.35 or 1.2 mGy/h. In our previous study (*8*), the mice were irradiated for 5 weeks, beginning at 7 weeks of age, and observed for 203 days. In the present study, this observation period was extended to the entire life span, and continuous irradiation was extended throughout the whole life span for the 0.35-mGy/h group and for the 1.2-mGy/h group. Figure 1 shows survival of these groups plotted as a function of time and clearly shows that the life span of the *lpr* mice was significantly prolonged by continuous low-dose-rate irradiation in a dose-rate-dependent manner ($P < 0.0001$ for b − c, d − e, b − d, c − e and b − e). Table 1 shows the 50% survival time for the *lpr* mice irradiated continuously at two different low dose rates for two different periods together with changes in immunological parameters at the two low dose rates. The prolongations of survival are in parallel with immunological activities, as discussed later.

Chronic Low-Dose-Rate γ Irradiation Suppressed Lymphadenopathy, Splenomegaly, and Kidney and Brain-Central Nervous System Syndromes

In parallel with the survival study, we examined the effects of continuous low-dose-rate γ irradiation on specific diseases in the *lpr* mice, i.e. lymphadenopathy (Figs. 2A and 3B), proteinuria (Fig. 2B) and splenomegaly (Fig. 3A). In the nonirradiated controls, these symptoms developed over time. However, the symptoms were suppressed re-

TABLE 1
50% Survival Time and Immunological Parameters for MRL-*lpr/lpr* Mice γ-Irradiated at Low Dose Rates

Group	Dose rate (mGy/h)	Irradiation period (week)	50% survival time (days)	Relative immunological parameters[a]			
				Thymus	Spleen		
				$CD4^+$ $CD8^+$ T cells	$CD8^+$ T cells	$CD3^+$ $B220^+$ cells	$B220^+$ $CD40^+$ cells
a	0	0	134[b]	1.0	1.0	1.0	1.0
b	0.35	5	159[c]	—	—	—	—
c	0.35	35	228	1.1	1.9	0.62	0.81
d	1.2	5	301[d]	—	—	—	—
e	1.2	74	502	1.2	2.2	0.45	0.37

[a] Measured in 12 mice in each group irradiated for 7 weeks beginning at 5 weeks of age and killed at 12 weeks of age.
[b] Identical results from previous (*8*) and present studies.
[c] Previous observation (*8*).
[d] Previous observation (*8*) was extended to whole life.

markably by continuous low-dose-rate irradiation, and this suppression was dependent on the dose rate. Furthermore, the severity of proteinuria in each mouse was reduced by the low-dose-rate irradiation (data not shown).

We previously demonstrated a remarkable amelioration of glomerulonephritis in the kidney and hemorrhage and inflammation in the brain in low-dose-rate-irradiated *lpr* mice using histological sections (*8*). In this study, we again observed histological amelioration of disease-specific damage to the kidney and the brain and also so observed that the whole brain, central nervous system, liver, intestine, lung, joints, blood vessels and skin in the low-dose-rate-irradiated mice appeared much less affected compared to the nonirradiated group (data not shown).

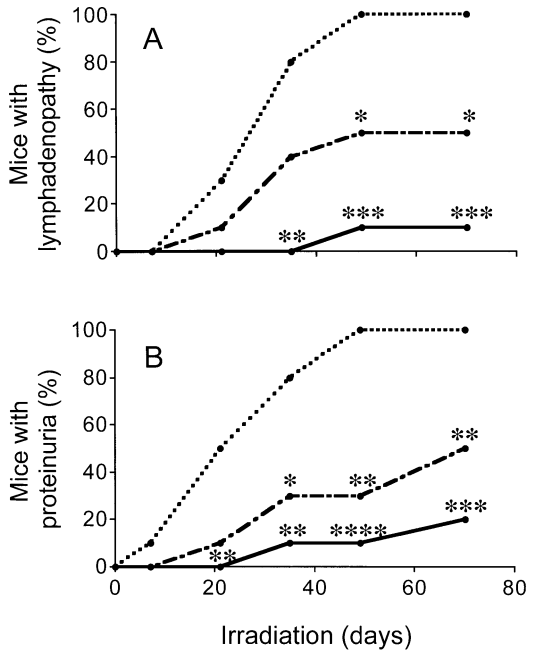

FIG. 2. Development of lymphadenopathy and proteinuria in MRL-*lpr/lpr* mice over time and its suppression by chronic γ irradiation at low dose rates beginning at 5 weeks of age. Upper curves: nonirradiated mice. Middle curves: mice irradiated with 0.35 mGy/h. Lower curves: mice irradiated with 1.2 mGy/h. $n = 10$ per point. Panel A: Mice with palpable inguinal lymph nodes. $*P < 0.01$, $**P < 0.001$, $***P < 0.0001$ compared to controls. Panel B: Mice with proteinuria over 30 mg/dl. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$ compared to controls.

Chronic Low-Dose-Rate γ Irradiation Increased Immune Activities

$CD4^+$ $CD8^+$ T cells in the thymus are very important for maturation of the immune system in the entire body. The population of these cells is small in *lpr* mice. However, chronic low-dose-rate irradiation significantly increased these cell populations (Fig. 4 and Table 1). $CD8^+$ T cells are important for the biological defense system. The cell population in the spleen was low in the nonirradiated mice. However, chronic low-dose-rate irradiation increased the cell population significantly (Fig. 5A and Table 1). $CD3^+$ CD45R/$B220^+$ ($CD3^+$ $B220^+$) cells are cells that attack the mouse's own organs and tissues (*37*). CD45R/$B220^+$ $CD40^+$ ($B220^+$ $CD40^+$) cells are characteristic in autoimmune diseases (*38*). The percentages of these cells in spleens are very high in the *lpr* mice. However, these high levels of abnormal cells were significantly decreased in the chronic low-dose-rate-irradiated *lpr* mice. Furthermore, suppressions of the abnormal cell populations were dependent on the dose rate (Fig. 5B, C and Table 1). Also noted was a tendency for an increase in the percentage of $CD4^+$ T cells in the spleen after chronic irradiation at a low dose rate, indicating augmentation of the immune system in the whole body. Furthermore, no changes in the total cell numbers in the bone marrow, no injury in the intestine, and no radiation-induced lymphomas were observed (data not shown).

DISCUSSION

The present report summarizes results obtained from lifelong observation of MRL-*lpr/lpr* mice that were continu-

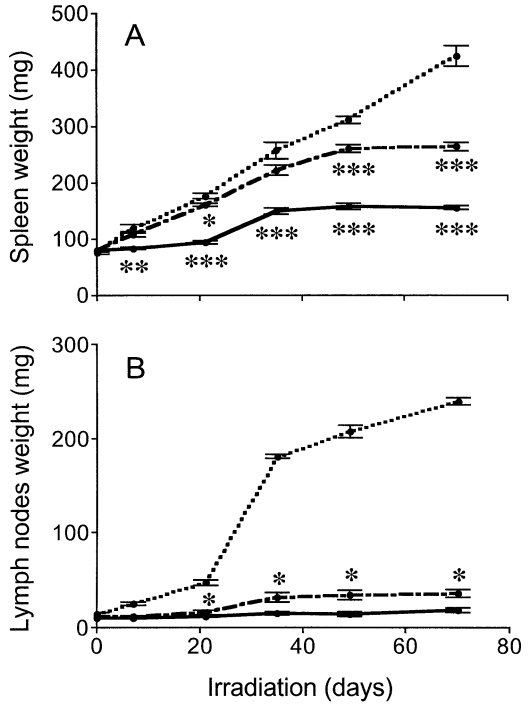

FIG. 3. Suppression of splenomegaly and lymphadenopathy in MRL-*lpr/lpr* mice by chronic γ irradiation at low dose rates from 5 weeks of age. Upper curves: nonirradiated mice. Middle curves: mice irradiated with 0.35 mGy/h. Lower curves: mice irradiated with 1.2 mGy/h. $n = 12$ per point. Panel A: The weights of the spleen. $^{*}P < 0.01$, $^{**}P < 0.001$, $^{***}P < 0.0001$ compared to controls. Panel B: The weights of the inguinal lymph nodes. $^{*}P < 0.0001$ compared to controls.

ously γ-irradiated at low dose rates. Previous observations (*8*) already indicated a prolongation of the life span of *lpr* mice accompanied by amelioration of disease and immune activation after 5 weeks of irradiation at low dose rates. The present study includes whole-life observation of these mice and provides information on the more profound ef-

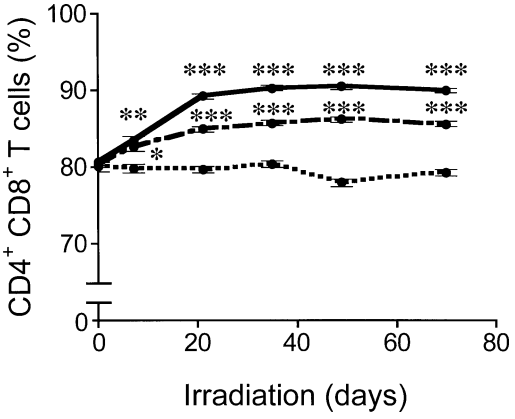

FIG. 4. Increases in normal immune cell populations in the thymuses of MRL-*lpr/lpr* mice by chronic γ irradiation at low dose rates from 5 weeks of age. Upper curve: mice irradiated with 1.2 mGy/h. Middle curve: mice irradiated with 0.35 mGy/h. Lower curve: nonirradiated mice. $n = 12$ per point. $^{*}P < 0.01$, $^{**}P < 0.001$, $^{***}P < 0.0001$ compared to controls.

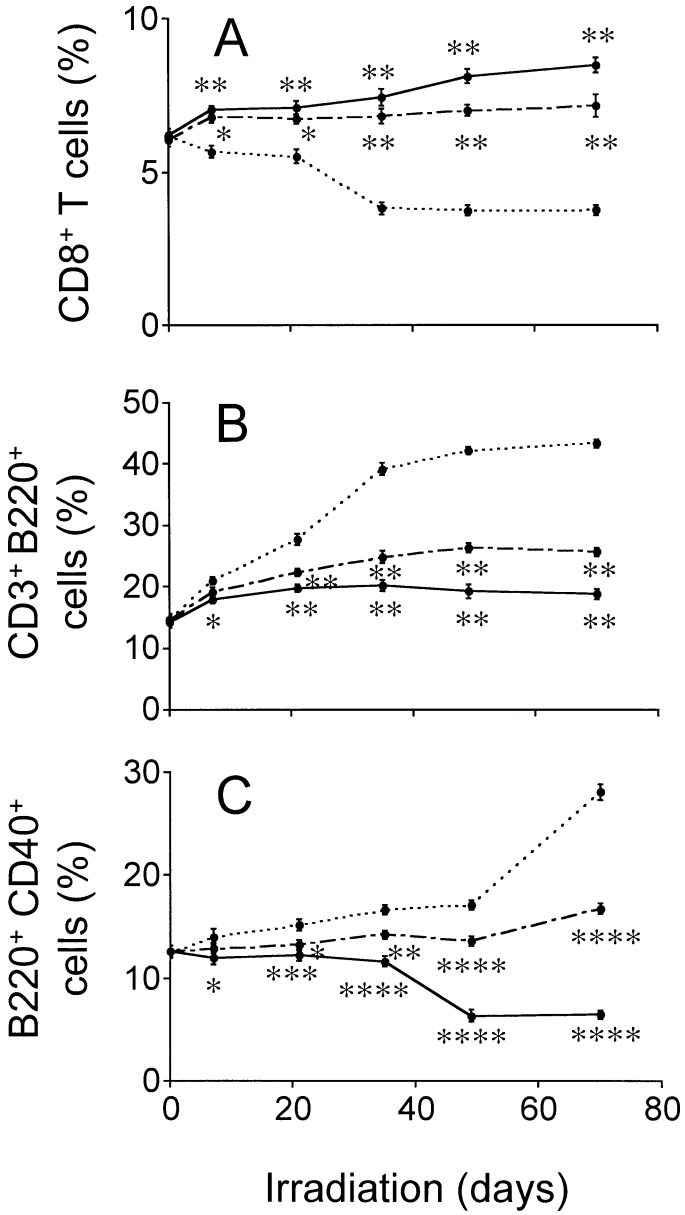

FIG. 5. Changes in immune cell populations in the spleens of MRL-*lpr/lpr* mice by chronic γ irradiation at low dose rates from 5 weeks of age. Solid curves: mice irradiated with 1.2 mGy/h. Dashed curves: mice irradiated with 0.35 mGy/h. Dotted curves: nonirradiated mice. $n = 12$ per point. Panel A: $CD8^+$ T cells. $^{*}P < 0.001$, $^{**}P < 0.0001$ compared to controls. Panel B: $CD3^+$ $B220^+$ cells. $^{*}P < 0.01$, $^{**}P < 0.0001$ compared to controls. Panel C: $B220^+$ $CD40^+$ cells. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$ compared to controls.

fects of life-long irradiation that accompany immune activation over time.

In this study, whole-life γ irradiation at low dose rates significantly prolonged the life span of *lpr* mice. The radiation suppressed total-body lymphadenopathy, splenomegaly and proteinuria together with kidney and brain-central nervous system syndromes, which are possible causes of death in these animals. It significantly increased $CD4^+$ $CD8^+$ T cells in the thymus and $CD8^+$ T cells in the spleen,

which indicates activation of the immune system, and decreased the representative abnormal cell populations, i.e. $CD3^+$ $B220^+$ cells and $B220^+$ $CD40^+$ cells. The $CD3^+$ $B220^+$ cells are autoreactive to the mouse's own organs and tissues throughout the body. The $B220^+$ $CD40^+$ cells are thought to be the major cell population in the progression of autoimmune diseases.

We believe that the immunological activations in the chronically low-dose-rate-irradiated mice are highly important in disease suppression in *lpr* mice. We believe that the increase in $CD8^+$ T cells may bring about an effective attack against abnormal cells and suppress the disease-specific abnormal cell populations, i.e. $CD3^+$ $B220^+$ cells and $B220^+$ $CD40^+$ cells. The $B220^+$ $CD40^+$ cells in the low-dose-rate-irradiated *lpr* mice decreased to the levels of those cells in normal mice without the *lpr* mutation, although the $CD3^+$ $B220^+$ cells still existed. We therefore consider that these decreases explain the amelioration of severe autoimmune diseases throughout the body.

It appeared that irradiation with 1.2 mGy/h was more effective than irradiation with 0.35 mGy/h in the prolongation of life and the enhancement of the immune system. We think it is necessary to irradiate mice at a higher dose rate for the full development of immunity and for suppression of these diseases. An optimum dose rate may exist beyond the dose-rate range employed in this study.

Furthermore, *lpr* mice spontaneously develop inflammation in the cerebral vessels, meninges and choroid plexus (*5, 6*). Our previous observations (*8*) showed that chronic low-dose-rate irradiation significantly suppressed cerebral and central nervous inflammation and hemorrhage in *lpr* mice, an observation that was confirmed in the present study (data not shown).

Pathways to apoptosis through non-Fas-mediated mechanisms may exist in chronically low-dose-rate-irradiated *lpr* mice. When Ootsuyama *et al.* (*28*) observed the effects of low-dose radiation in the amelioration of disease in MRL-*gld/gld* mice, which have a mutation in the Fas ligand, they explained the effects of such radiation by recovery of the apoptotic activity as indicated in spleen cells. However, in this study, we found no significant difference between the nonirradiated control mice and the low-dose-rate-irradiated mice in terms of the percentage of thymocytes stained by propidium iodide, trypan blue and erythrosin B, respectively, and the expression of phosphatidylserine on the thymocytes. Furthermore, there was no expression of Fas, the apoptosis-regulating molecule, on the thymocytes, splenocytes and lymph node cells (data not shown).

The difference between *lpr* and *gld* mutations or between the dose rates of radiation in the experiments of Ootsuyama *et al.* (*28*) and our experiments may explain the difference in apoptosis. Therefore, we consider the immunological modification in the low-dose-rate-irradiated mice to be the primary reason for increased survival through amelioration of diseases, although changes in non-Fas-mediated apoptotic activity might be indirectly involved.

Finally, it is intriguing that immunological activation by low-dose-rate irradiation may be effective in suppressing tumor induction. Indeed, we have found suppression of radiation-induced thymic lymphomas in C57BL/6 mice to less than half (40%) by continuous low-dose-rate γ irradiation under conditions identical to those in the present study (*39*).

In summary, we found ameliorations of multiple severe diseases, including total-body lymphadenopathy, splenomegaly and serious autoimmune diseases throughout the body, including kidney and brain-central nervous system syndromes, in MRL-*lpr/lpr* mice chronically irradiated at low dose rates. The life span of the mice was prolonged remarkably in parallel with the activation of the immune system without severe tissue damage. Thus chronic low-dose-rate irradiation is expected to be useful in the treatment of certain lymphomas and autoimmune diseases.

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REFERENCES

1. E. D. Murphy and J. B. Roths, Autoimmunity and lymphoproliferation: Induction by mutant gene *lpr,* and acceleration by a male-associated factor in strain BXSB mice. In *Genetic Control of Autoimmune Disease* (N. R. Rose, P. E. Bigazzi and N. L. Warner, Eds.), pp. 207–221. Elsevier, Amsterdam, 1978.
2. B. S. Andrews, R. A. Eisenberg, A. N. Theofilopoulos, S. Izui, C. B. Wilson, P. J. McConahey, E. D. Murphy, J. B. Roths and F. J. Dixon, Spontaneous murine lupus-like syndromes: Clinical and immunopathological manifestations in several strains. *J. Exp. Med.* **148**, 1198–1215 (1978).
3. E. D. Murphy and J. B. Roths, A Y chromosome associated factor in strain BXSB producing accelerated autoimmunity and lymphoproliferation. *Arthritis Rheum.* **22**, 1188–1194 (1979).
4. J. L. Chu, J. Drappa, A. Parnassa and K. B. Elkon, The defect in *Fas* mRNA expression in MRL/*lpr* mice is associated with insertion of the retrotransposon, *ETn. J. Exp. Med.* **178**, 723–730 (1993).
5. E. L. Alexander, E. D. Murphy, J. B. Roths and G. E. Alexander, Congenic autoimmune murine models of central nervous system disease in connective tissue disorders. *Ann. Neurol.* **14**, 242–248 (1983).
6. E. L. Alexander, C. Moyer, G. S. Travlos, J. B. Roths and E. D. Murphy, Two histopathologic types of inflammatory vascular disease in MRL/Mp autoimmune mice. Model for human vasculitis in connective tissue disease. *Arthritis Rheum.* **28**, 1146–1155 (1985).
7. T. Watanabe, Y. Sakai, S. Miyawaki, A. Shimizu, O. Koiwai and K. Ohno, A molecular genetic linkage map of mouse chromosome 19, including the *lpr, Ly-44,* and *Tdt* genes. *Biochem. Genet.* **29**, 325–335 (1991).
8. Y. Ina and K. Sakai, Prolongation of life span associated with immunological modification by chronic low-dose-rate irradiation in MRL-*lpr/lpr* mice. *Radiat. Res.* **161**, 168–173 (2004).
9. R. E. Anderson and I. Lefkovits, *In vitro* evaluation of radiation-induced augmentation of the immune response. *Am. J. Pathol.* **97**, 456–472 (1979).
10. S. Z. Liu, W. H. Liu and J. B. Sun, Radiation hormesis: Its expression in the immune system. *Health Phys.* **52**, 579–583 (1987).

11. S. J. James and T. Makinodan, T cell potentiation in normal and autoimmune-prone mice after extended exposure to low doses of ionizing radiation and/or caloric restriction. *Int. J. Radiat. Biol.* **53**, 137–152 (1988).

12. S. Z. Liu, Radiation hormesis. A new concept in radiological science. *Chin. Med. J.* **102**, 750–755 (1989).

13. S. J. James, S. M. Enger, W. J. Peterson and T. Makinodan, Immune potentiation after fractionated exposure to very low doses of ionizing radiation and/or caloric restriction in autoimmune-prone and normal C57Bl/6 mice. *Clin. Immunol. Immunopathol.* **55**, 427–437 (1990).

14. J. Matsubara, V. Turcanu, P. Poindron and Y. Ina, Immune effects of low-dose radiation: Short-term induction of thymocyte apoptosis and long-term augmentation of T-cell-dependent immune responses. *Radiat. Res.* **153**, 332–338 (2000).

15. S. Kojima, S. Matsumori, H. Ishida and K. Yamaoka, 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 (2000).

16. S. Kojima, H. Ishida, M. Takahashi and K. Yamaoka, Elevation of glutathione induced by low-dose gamma rays and its involvement in increased natural killer activity. *Radiat. Res.* **157**, 275–280 (2002).

17. Y. Ina and K. Sakai, Immunological network activation by low-dose rate irradiation: Analysis of cell populations and cell surface molecules in whole body irradiated mice. In *Molecular Mechanisms for Radiation-induced Cellular Response and Cancer Development* (K. Tanaka, T. Takabatake, K. Fujikawa, T. Matsumoto and F. Sato, Eds.), pp. 227–233. Art Press, Aomori, 2003.

18. H. J. Dobbs, A. Barrett, A. Y. Rostom and M. J. Peckham, Total-body irradiation in advanced non-Hodgkin's lymphoma. *Br. J. Radiol.* **54**, 878–881 (1981).

19. P. Jacobs and H. S. King, A randomized prospective comparison of chemotherapy to total body irradiation as initial treatment for the indolent lymphoproliferative diseases. *Blood* **69**, 1642–1646 (1987).

20. R. N. Shen, L. Lu, H. E. Kaiser and H. E. Broxmeyer, Murine AIDS cured by low dosage total body irradiation. *Adv. Exp. Med. Biol.* **407**, 451–458 (1997).

21. S. Hashimoto, H. Shirato, M. Hosokawa, T. Nishioka, Y. Kuramitsu, K. Matsushita, M. Kobayashi and K. Miyasaka, The suppression of metastases and the change in host immune response after low-dose total-body irradiation in tumor-bearing rats. *Radiat. Res.* **151**, 717–724 (1999).

22. M. Takahashi, S. Kojima, K. Yamaoka and E. Niki, Prevention of type I diabetes by low-dose gamma irradiation in NOD mice. *Radiat. Res.* **154**, 680–685 (2000).

23. E. Lorenz, J. W. Hollcroft, E. Miller, C. C. Congdon and R. Schweisthal, Long-term effects of acute and chronic irradiation in mice. I. Survival and tumor incidence following chronic irradiation of 0.11 r per day. *J. Natl. Cancer Inst.* **15**, 1049–1058 (1955).

24. H. S. Ducoff, Form of the increased longevity of *Tribolium* after X-irradiation. *Exp. Gerontol.* **10**, 189–193 (1975).

25. M. Mine, Y. Okumura, M. Ichimaru, T. Nakamura and S. Kondo, Apparently beneficial effect of low to intermediate doses of A-bomb radiation on human lifespan. *Int. J. Radiat. Biol.* **58**, 1035–1043 (1990).

26. R. E. J. Mitchel, J. S. Jackson, R. A. McCann and D. R. Boreham, The adaptive response modifies latency for radiation-induced myeloid leukemia in CBA/H mice. *Radiat. Res.* **152**, 273–279 (1999).

27. R. E. J. Mitchel, J. S. Jackson, D. P. Morrison and S. M. Carlisle, Low doses of radiation increase the latency of spontaneous lymphomas and spinal osteosarcomas in cancer-prone, radiation-sensitive *Trp53* heterozygous mice. *Radiat. Res.* **159**, 320–327 (2003).

28. A. Ootsuyama, R. Okazaki and T. Norimura, Effect of extended exposure to low-dose radiation on autoimmune diseases of immunologically suppressed MRL/MpTn-*gld/gld* mice. *J. Radiat. Res.* **44**, 243–247 (2003).

29. R. Watanabe-Fukunaga, C. I. Brannan, N. G. Copeland, N. A. Jenkins and S. Nagata, Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis. *Nature* **356**, 314–317 (1992).

30. J. B. Roths, E. D. Murphy and E. M. Eicher, A new mutation, *gld*, that produces lymphoproliferation and autoimmunity in C3H/HeJ mice. *J. Exp. Med.* **159**, 1–20 (1984).

31. W. F. Davidson, F. J. Dumont, H. G. Bedigian, B. J. Fowlkes and H. C. Morse, III, Phenotypic, functional, and molecular genetic comparisons of the abnormal lymphoid cells of C3H-*lpr/lpr* and C3H-*gld/gld* mice. *J. Immunol.* **136**, 4075–4084 (1986).

32. M. Nose, M. Nishimura and M. Kyogoku, Analysis of granulomatous arteritis in MRL/Mp autoimmune disease mice bearing lymphoproliferative genes. The use of mouse genetics to dissociate the development of arteritis and glomerulonephritis. *Am. J. Pathol.* **135**, 271–280 (1989).

33. P. L. Cohen and R. A. Eisenberg, *Lpr* and *gld*: Single gene models of systemic autoimmunity and lymphoproliferative disease. *Annu. Rev. Immunol.* **9**, 243–269 (1991).

34. M. L. Watson, P. D'Eustachio, B. A. Mock, A. D. Steinberg, H. C. Morse, III, R. J. Oakey, T. A. Howard, J. M. Rochelle and M. F. Seldin, A linkage map of mouse chromosome 1 using an interspecific cross segregating for the *gld* autoimmunity mutation. *Mamm. Genome* **2**, 158–171 (1992).

35. T. Takahashi, M. Tanaka, C. I. Brannan, N. A. Jenkins, N. G. Copeland, T. Suda and S. Nagata, Generalized lymphoproliferative disease in mice, caused by a point mutation in the Fas ligand. *Cell* **76**, 969–976 (1994).

36. Y. Hoshi, T. Nomura, T. Oda, T. Iwasaki, K. Fujita, T. Ishikawa, A. Kato, K. Sakai, H. Tanooka and T. Yamada, Application of a newly developed photoluminescence glass dosimeter for measuring the absorbed dose in individual mice exposed to low-dose rate ^{137}Cs γ-rays. *J. Radiat. Res.* **41**, 129–137 (2000).

37. C. Diaz-Gallo and V. R. Kelley, Self-regulation of autoreactive kidney-infiltrating T cells in MRL-lpr nephritis. *Kidney Int.* **44**, 692–699 (1993).

38. C. Mohan, Y. Shi, J. D. Laman and S. K. Datta, Interaction between CD40 and its ligand gp39 in the development of murine lupus nephritis. *J. Immunol.* **154**, 1470–1480 (1995).

39. Y. Ina, H. Tanooka, T. Yamada and K. Sakai, Suppression of thymic lymphoma induction by life-long low-dose-rate irradiation accompanied by immune activation in C57BL/6 mice. *Radiat. Res.* **163**, 153–158 (2005).