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journal or publication title	Dose-Response
volume	16
number	3
page range	1559325818790152
year	2018-08-20
Publisher	SAGE Publications
Rights	(C) 2018 The Author(s).?
Author's flag	publisher
URL	http://id.nii.ac.jp/1394/00000792/

doi: info:doi/10.1177/1559325818790152

Increased Hematopoietic Stem Cells/ Hematopoietic Progenitor Cells Measured as Endogenous Spleen Colonies in Radiation-Induced Adaptive Response in Mice (Yonezawa Effect)

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Abstract

The existence of radiation-induced adaptive response (AR) was reported in varied biosystems. In mice, the first in vivo AR model was established using X-rays as both the priming and the challenge doses and rescue of bone marrow death as the end point. The underlying mechanism was due to the priming radiation-induced resistance in the blood-forming tissues. In a series of investigations, we further demonstrated the existence of AR using different types of ionizing radiation (IR) including low linear energy transfer (LET) X-rays and high LET heavy ion. In this article, we validated hematopoietic stem cells/hematopoietic progenitor cells (HSCs/HPCs) measured as endogenous colony-forming units-spleen (CFU-S) under AR inducible and uninducible conditions using combination of different types of IR. We confirmed the consistency of increased CFU-S number change with the AR inducible condition. These findings suggest that AR in mice induced by different types of IR would share at least in part a common underlying mechanism, the priming IR-induced resistance in the blood-forming tissues, which would lead to a protective effect on the HSCs/HPCs and play an important role in rescuing the animals from bone marrow death. These findings provide a new insight into the mechanistic study on AR in vivo.

Keywords

adaptive response, ionizing radiation, heavy ion, colony forming units-spleen, mice

Introduction

Ionizing radiation (IR) at high doses is detrimental to the exposed organism, and exposure to IR could increase the risk of developing cancers and other health-related problems even including acute radiation syndrome such as bone marrow death. However, biological effects of IR at low dose or low dose rate remain elusive. On the other hand, adaptation to the environmental genotoxic stresses or insults, such as IR, is one of the fundamental characteristics of life. In particular, prior mild stresses can provide some aid to prepare organisms for subsequent more severe stresses.¹ Radiation-induced adaptive response (AR) is a phenomenon that a priming low dose

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Received 2 May 2018; received revised 1 June 2018; accepted 12 June 2018

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of IR induces resistance to a subsequent challenge exposure to IR at higher doses, and its studies could provide important scientific basis for IR risk estimates, protection, and practical applications.² Since the editio princeps of AR concept introduced into radiation biology,³ it has been demonstrated in a variety of in vitro, in utero, and in vivo systems with end points such as DNA damage, chromosomal aberrations, cell transformation, cell death, and mutation in the in vitro experiments and prenatal death, malformation, hematopoietic death, and carcinogenesis.^{1,4-6}

High atomic number and energy (HZE) particles such as carbon, oxygen, silicon, and iron are important component of space radiation, from the solar particle events and the galactic cosmic rays.^{7,8} Compared to photon and proton radiation, HZE particles bearing higher energy could cause both acute and long-term damage to bone marrow via increased production of reactive oxygen species, showing stronger detrimental effects (with higher relative biological effectiveness) on the hematopoietic system including decreased peripheral blood counts and reduced hematopoietic stem cells (HSCs) and progenitor cells (HPCs) in laboratory animal models⁹⁻²⁰ and, in addition, raising hematological cancer risk via bone marrow cell reprogramming.²¹

The AR mouse model for rescuing bone marrow death established by Yonezawa and colleagues^{1,22-24} was repeatedly verified.^{25,26} This model was named generally as “Yonezawa Effect” in Japan, which was originally established using low linear energy transfer (LET) X-rays as both the priming and the challenge IR, with the underlying mechanism that induction of radioresistance in blood forming tissues by the priming IR rescued the bone marrow death caused by the challenge high dose. In a series of investigations in our laboratory, we first verified and confirmed the existence of AR in mice using low-LET X-rays to deliver both the priming and the challenge IR and the 30-day survival test to estimate the efficacy for rescuing bone marrow death. Then, we further demonstrated the existence of AR using low-LET X-rays as the priming IR and high-LET heavy ion IR (carbon, neon, and silicon particles) as the challenge IR. Recently, we showed that, in this model, AR could be induced by high-LET heavy ion IR (carbon particles) as the priming IR and low-LET X-rays or high-LET heavy ion IR (carbon and neon particles) as the challenge IR.²⁰⁻²⁹ In the present work, we validated the HSCs/HPCs measured as endogenous colony-forming units-spleen (CFU-S) under AR inducible and uninducible conditions using combination of low-LET X-rays and high-LET heavy ion IR as priming and challenge IR. By verifying the number of CFU-S under different conditions, 12 days after the animals received the challenge IR, the present investigation was aimed to study the recovery of HSCs/HPCs in this AR mouse model. We confirmed the consistency of CFU-S number with the AR induction conditions: significantly increased number of CFU-S under AR inducible conditions and no markedly increased number of CFU-S under AR uninducible conditions.

Materials and Methods

Animals

Five-week-old C57BL/6J Jms strain female mice were purchased from SLC, Inc (Japan) and maintained in a conventional animal facility under a 12 hour light–12 hour dark photoperiod. The animals were housed in autoclaved aluminum cages with sterilized wood chips and allowed to access standard laboratory chow (MB-1; Funabashi Farm Co, Japan) and acidified water ad libitum. The animals were acclimatized to the laboratory conditions for 1 week before use. To avoid possible effects from the developmental condition of the animals, 6-week-old mice with a significantly different body weight (more or less than the mean + 2 SD) were omitted from this study. Based on our preliminary trials, in the present study, at least 6 mice were used in each experimental point. All experimental protocols involving mice were reviewed and approved by The Institutional Animal Care and Use Committee of the National Institute of Radiological Sciences (NIRS). The experiments were performed in strict accordance with the NIRS *Guidelines for the Care and Use of Laboratory Animals*.

Irradiation

For low-LET IR, X-rays were generated with an X-ray machine (Pantak-320S; Shimadzu, Japan) operated at 200 kVp and 20 mA, using a 0.50 mm Al + 0.50 mm Cu filter. An exposure-rate meter (AE-1321M; Applied Engineering Inc, Japan) with an ionization chamber (C-110, 0.6 ml, JARP, Applied Engineering Inc, Japan) was used for the dosimetry. The dose rate for delivering the priming dose and the challenge dose was at about 0.30 Gy/min and 0.90 Gy/min, respectively. For high-LET heavy ion IR, the monoenergetic ion beam of carbon, neon, and iron particles was generated and accelerated by a synchrotron, the Heavy Ion Medical Accelerator in Chiba at NIRS, Japan. The beam energy was 290 MeV/nucleon, 400 MeV/nucleon, and 500 MeV/nucleon for carbon, neon, and iron particles, corresponding to an average LET value of about 15 keV/μm, 30 keV/μm, and 200 keV/μm, respectively. The dose rate was at about 0.10 Gy/min and 2.00 Gy/min for delivery of the priming dose and the challenge dose, respectively. The mice held in acrylic containers were exposed to total body irradiation at room temperature.

Mouse Model for Radiation-Induced AR

The AR mouse model for rescue of bone marrow death and study on increase in the number of CFU-S established by Yonezawa and colleagues^{24,30} was adopted, verified, and confirmed under the experimental conditions in our research facilities and finally applied to a series of our investigations using both low-LET X-rays and high-LET particles. The timing for delivery of the priming dose and challenge dose was on postnatal ages of 6 and 8 weeks of the mice, respectively. The present work was a part of the investigations focusing on the recovery of HSCs/HPCs under AR. The AR inducible and uninducible

Table 1. Efficacy for Induction of AR by Combination of Different Types of IR in Mice.

Dose, Gy	Challenge IR	Thirty-day Survival, %		
		Challenge IR	Priming + Challenge IR	AR Induction
Priming IR				
X-rays (0.50)	X-rays (7.50)	16.7	83.3	Yes
X-rays (0.50)	C (6.50)	15.0	66.7	Yes
X-rays (0.50)	Fe (6.00)	23.3	26.7	No
C (0.45)	X-rays (7.50)	16.7	73.3	Yes
Fe (0.45)	X-rays (7.50)	16.7	0.0	No
C (0.45)	C (6.50)	10.0	36.7	Yes
C (0.45)	Ne (5.50)	21.6	70.0	Yes
C (0.45)	Fe (5.75)	80.0	90.0	No

Abbreviations: AR, adaptive response; IR, ionizing radiation.

conditions obtained in our previous studies for rescue of bone marrow death¹⁹⁻²⁷ are summarized (Table 1). In Table 1, the “Yes” for AR induction meant a successful AR induction as judged by a significant increase in the animal survival after receiving the priming radiation prior to the challenge radiation in the 30-day survival test, and the “No” for no significant increase in the survival was induced in the presence of the priming radiation. To look for suitable experimental conditions, especially both the challenge dose altitude of each type of IR and the timing that makes the CFU-S more distinctly observable, 3 to 4 sublethal doses for the challenge IR of each type in each combination of the priming and the challenge exposure were validated in preliminary trials, and the doses listed in Table 1 were finally used. In brief, the combination exposures were (1) the priming dose was 0.50 Gy for low-LET X-rays and 0.45 Gy for high-LET carbon or iron particles; (2) the challenge dose for X-rays was 5.00 Gy in the combined exposure to priming X-rays and 4.75 Gy to priming carbon or iron particles; (3) the challenge dose for carbon particles was 5.00 Gy and 5.25 Gy following the priming IR from X-rays and carbon particles, respectively; and (4) the challenge dose was 5.00 Gy and 5.5 Gy for neon particles and iron particles, respectively.

Enumeration of Endogenous CFU-S

To evaluate the number of HSCs/HPCs in vivo in mice under experimental conditions capable or incapable of inducing AR, the method established by Till and McCulloch,³¹ bearing advantages over the exogenous transplant system, including simplicity and rapidity, avoiding in vitro cell manipulation,^{32,33} was adopted and applied to the present study. In brief, the mice were killed 11, 12, or 13 days after receiving the challenge dose. The spleens were removed, weighed, and then fixed in Bouin fixative (having a mixing ratio 15:5:1 of saturated picric acid, formaldehyde, and glacial acetic acid; purchased from Wako Pure Chemical Industries, Ltd, Japan) for 24 hours. The macroscopic nodules or colonies on the surface of the organ were counted as endogenous CFU-S using a stereoscopic microscope (Nikon SMZ-10; Nikon Instech Co, Ltd, Japan)

at 10× magnification. Timing of spleen sample collection was according to that the numeration of endogenous CFU-S formed 11 days or later after irradiation provides a measure of viable pluripotent HSCs/HPCs,^{31,34} and longer intervals were avoided due to the colonies fused together, preventing scoring of individual colonies. Based on the ease for clear distinction of individual colonies, data obtained on 12 days after the challenge IR were used to judge the consistency of CFU-S number change and AR induction. Six to 12 animals were used per experimental point.

Statistical Analysis

Statistical evaluation of the data was done using the Student *t* test, and the statistical significance was assigned to $P < .05$.

Results

Verification of the Radiation-Induced AR Mouse Model Using CFU-S as the End Point

Reproducibility of the radiation-induced AR mouse model (Yonezawa Effect) using CFU-S as the end point³⁰ was verified under the experimental setup in the present study. Under the AR inducible conditions, the animals were total body irradiated with a priming dose of 0.50 Gy X-rays at postnatal 6 weeks followed by a challenge dose of 5.00 Gy X-rays at postnatal 8=weeks. Under the AR uninducible conditions, the animals were total body irradiated with only a challenge dose of 5.00 Gy X-rays at postnatal 8 weeks. The number of CFU-S was measured on 11, 12, and 13 days after the challenge IR. Results showed that the priming dose markedly increased the mean number of CFU-S from 3.2 to 11.8, 4.0 to 36.2, and 5.6 to 50.2 on the days 11, 12, and 13, respectively, after the challenge IR (Figure 1). Results clearly indicated that AR was induced with efficient reliability and reproducibility in our experimental setup using the number of CFU-S as the end point. Serving also as a positive control, the verification work was performed in parallel to the following investigations using combination of different types of IR.

Validation of CFU-S in AR Induced by Priming IR From Low-LET X-Rays and Challenge IR From High-LET Particles

The CFU-S assay was performed to validate whether significant increase in the number of CFU-S occurred in the animals under AR inducible condition (exposure of a priming dose of 0.50 Gy X-rays at postnatal 6 weeks followed by a challenge dose of 5.00 Gy carbon particles at postnatal 8 weeks) and the AR uninducible condition (the animals irradiated with only the challenge dose; the animals irradiated with a priming dose of 0.50 Gy X-rays at postnatal 6 weeks followed by a challenge dose of 5.50 Gy iron ions). The mean number of CFU-S was significantly increased from 7.8 to 15.0, 15.9 to 28.6, and 24.3 to 44.0, respectively, on 11, 12, and 13 days after the challenge

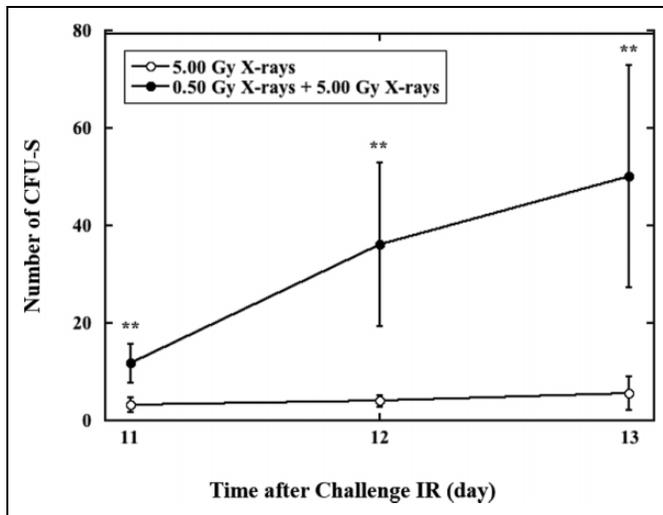


Figure 1. Verification and confirmation of adaptive response in mice (Yonezawa Effect) induced by low-LET X-rays as both priming and challenge ionizing radiation (IR) using colony-forming units-spleen (CFU-S) as the end point. Effect of a priming dose of 0.50 Gy X-rays on a subsequent challenge dose of 5.00 Gy X-rays on the number of CFU-S was verified. Under the adaptive response (AR) inducible condition, the animals were total body irradiated with a priming dose of 0.50 Gy X-rays at postnatal 6 weeks and then followed by a challenge dose of 5.00 Gy X-rays at postnatal 8 weeks (closed circles with solid line). Under the AR uninducible condition, the animals were total body irradiated with only the challenge dose (open circles with solid line). The number of CFU-S was measured on the days 11, 12, and 13 after the challenge IR. Data of each experimental point were from 6 to 12 mice. Two asterisks (**) indicate statistically significant differences ($P < .01$) between the 2 groups that were compared.

IR (Figure 2A). On the other hand, no increased number of CFU-S was observed in the animals receiving both the priming X-rays and the challenge iron IR when compared to the animals receiving only the challenge iron IR (Figure 2B). On the 11th day after the challenge iron IR, the mean number of CFU-S was even markedly higher in the animals receiving only the challenge iron IR when compared to the animals receiving both the priming X-rays and the challenge iron IR (Figure 2B), indicating an additive effect on reducing CFU-S from the combined exposure. These results clearly showed that under AR inducible condition when the priming IR was from low LET X-rays and the challenge IR was from high LET carbon particles, increased number of CFU-S was confirmed. On the other hand, under AR uninducible condition, when the priming IR was from low-LET X-rays and the challenge IR was from high-LET iron particles, no increased number of CFU-S was confirmed.

Validation of CFU-S in AR Induced by Priming IR From High-LET Particles and Challenge IR From Low-LET X-Rays

The CFU-S assay was performed to validate whether significant increase in the number of CFU-S occurred under AR inducible condition (ie, the animals irradiated with a priming

dose of 0.45 Gy carbon ions at postnatal 6 weeks followed by a challenge dose of 4.75 Gy X-rays at postnatal 8 weeks vs the animals receiving only the challenge dose of 4.75 Gy X-rays at postnatal 8 weeks) and the AR uninducible condition (ie, the animals irradiated with 0.45 Gy iron ions at postnatal 6 weeks followed by a challenge dose of 4.75 Gy X-rays at postnatal 8 weeks vs the animals receiving only the challenge dose of 4.75 Gy X-rays at postnatal 8 weeks). On the 11th day after the challenge IR, difference in the mean number of CFU-S was not statistically significant between the animals irradiated with a priming dose of 0.45 Gy carbon particles followed by a challenge dose of 4.75 Gy X-rays and the animals receiving only the challenge dose of 4.75 Gy X-rays (Figure 3A). The mean number of CFU-S was significantly increased from 6.7 to 22.3 and 4.2 to 32.5, respectively, on 12 and 13 days after the challenge IR (Figure 3A). On the other hand, no increased mean number of CFU-S was observed in the animals receiving both the priming dose from iron particles followed by the challenge dose from X-rays and the animals irradiated with only the challenge dose from X-rays (Figure 3B). These results clearly showed that under AR inducible condition when the priming IR was from high-LET carbon particles and the challenge IR was from low-LET X-rays, increased mean number of CFU-S was confirmed. Under AR uninducible condition, when the priming IR was from high LET iron particles and the challenge IR was from low LET X-rays, no increased mean number of CFU-S was observed.

Validation of CFU-S in AR Induced by High-LET Particles as Both Priming IR and Challenge IR

The CFU-S assay was performed to validate whether significant increase in the number of CFU-S occurred under AR inducible condition (ie, the animals irradiated with a priming dose of 0.45 Gy carbon particles at postnatal 6 weeks followed by a challenge dose of 5.25 Gy carbon particles or 5.00 Gy neon particles at postnatal 8 weeks vs the animals irradiated with only the challenge dose) and the AR uninducible condition (ie, the animals irradiated with a priming dose of 0.45 Gy carbon particles at postnatal 6 weeks followed by a challenge dose of 5.50 Gy iron particles at postnatal 8 weeks). On 11, 12, and 13 days after the challenge IR, the mean number of CFU-S was significantly increased from 2.6 to 9.2, 7.0 to 22.4, and 15.5 to 43.0 for the animals receiving the combined exposure to carbon particles when compared to those receiving only the challenge IR from carbon particles (Figure 4A); the mean number of CFU-S was from 1.3 to 2.8, 4.0 to 8.6, and 4.7 to 10.1 for the animals exposed to the priming IR from carbon particles followed by the challenge IR from neon particles, and the increase was statistically significant on the 12th day (Figure 4B). On the other hand, no increased mean number of CFU-S was observed in the animals receiving both the priming dose from carbon particles followed by the challenge dose from iron particles when compared to those receiving only the challenge dose from iron particles (Figure 4C). These results clearly showed that under AR inducible condition when the priming

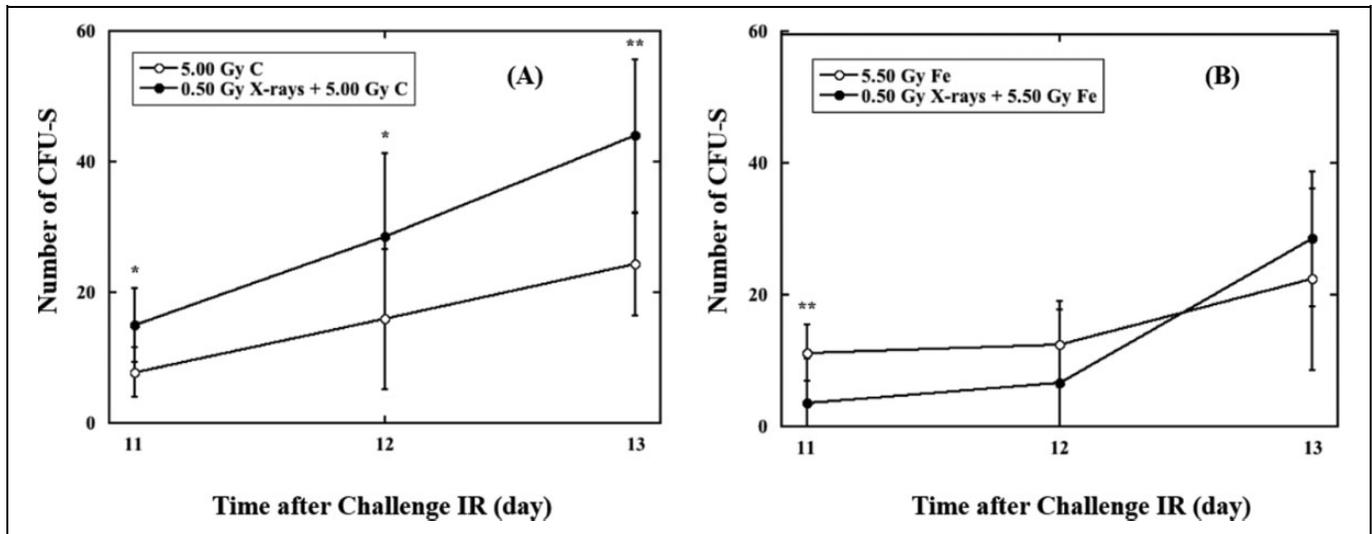


Figure 2. Validation of adaptive response (AR) in mice (Yonezawa Effect) induced by low LET X-rays as the priming ionizing radiation (IR) and high LET particles as the challenge IR using colony forming units-spleen (CFU-S) as the end point. Effect of a priming dose of 0.50 Gy X-rays on a subsequent challenge dose from carbon ions (A) or iron ions (B) on the number of CFU-S was verified. Under the AR inducible condition, the animals were total body irradiated with a priming dose of 0.50 Gy X-rays at postnatal 6 weeks, and then followed by a challenge dose of 5.00 Gy carbon ions (A) at postnatal 8 weeks. Under the AR uninducible condition, (1) the animals were total body irradiated with a priming dose of 0.50 Gy X-rays at postnatal 6 weeks, and then followed by a challenge dose of 5.50 Gy iron ions (B), and (2) the animals were only total body irradiated with the challenge dose. Closed circles with solid line stand for the groups receiving both the low dose and the high dose at postnatal 6 weeks and 8 weeks, respectively. Open circles with solid line stand for the groups receiving only the challenge dose at postnatal 8 weeks. The number of CFU-S was measured on the days 11, 12, and 13 after the challenge IR. Data of each experimental point were from 6 to 12 mice. One asterisk (*) stands of statistically significant differences ($P < .05$) between the 2 groups that were compared. Two asterisks (***) indicate statistically significant differences ($P < .01$) between the 2 groups that were compared. C stands for carbon and Fe stands for iron.

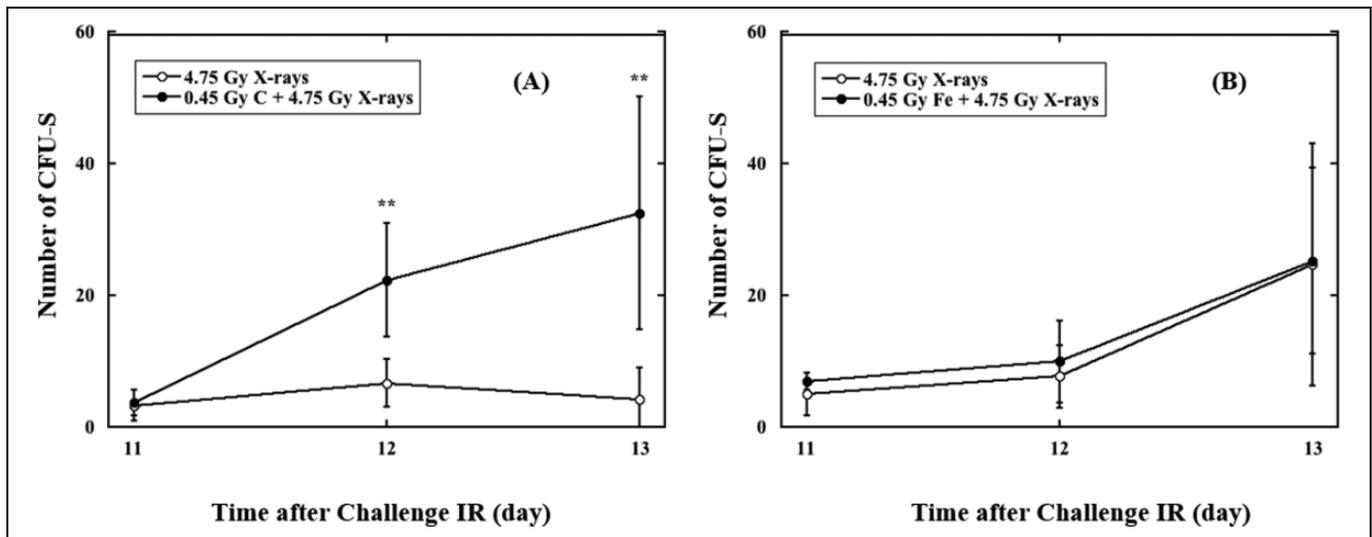


Figure 3. Validation of adaptive response in mice (Yonezawa Effect) induced by high-LET particles as the priming ionizing radiation (IR) and low-LET X-rays as the challenge IR using colony forming units-spleen (CFU-S) as the end point. Effect of a priming dose of 0.45 Gy carbon ions (A) or 0.45 Gy iron ions (B) on a subsequent challenge dose of 4.75 Gy from X-rays on the number of CFU-S was verified. Under the adaptive response (AR) inducible condition, the animals were total body irradiated with a priming dose of 0.45 Gy carbon ions at postnatal 6 weeks, and then followed by a challenge dose of 4.75 Gy X-rays at postnatal 8 weeks. Under the AR uninducible condition, the animals were total body irradiated with 0.45 Gy iron ions at postnatal 6 weeks, and then followed by a challenge dose of 4.75 Gy X-rays at postnatal 8 weeks. Closed circles with solid line stand for the groups receiving both the priming dose and the challenge dose at postnatal 6 weeks and postnatal 8 weeks, respectively. Open circles with solid line stand for the groups receiving only the challenge dose at postnatal 8 weeks. The number of CFU-S was measured on the days 11, 12, and 13 after the challenge IR. Data of each experimental point were from 6 to 12 mice. Two asterisks (***) indicate statistically significant differences ($P < .01$) between the 2 groups that were compared. C stands for carbon and Fe stands for iron.

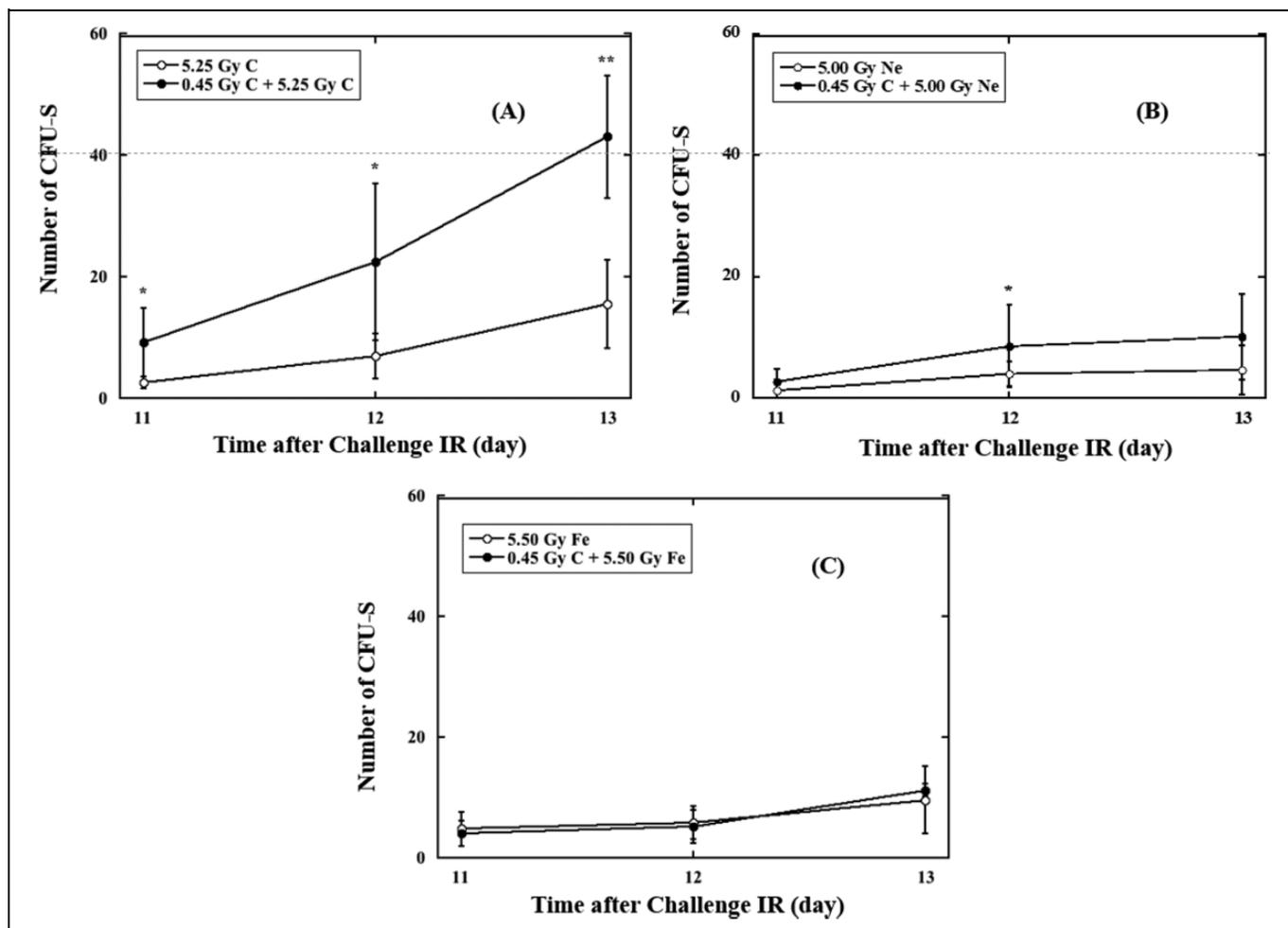


Figure 4. Validation of adaptive response in mice (Yonezawa Effect) induced by high-LET particles as both the priming ionizing radiation (IR) and the challenge IR using colony-forming units-spleen (CFU-S) as the end point. Effect of a priming dose of 0.45 Gy carbon ions on a subsequent challenge dose from carbon ions (A), neon ions (B), or iron ions (C) on the number of CFU-S was verified. Under the adaptive response (AR) inducible condition, the animals were total body irradiated with a priming dose of 0.45 Gy carbon ions at postnatal 6 weeks, and then followed by a challenge dose of 5.25 Gy carbon ions or of 5.00 Gy neon ions at postnatal 8 weeks. Under the AR uninducible condition, (1) the animals were total body irradiated with a priming dose of 0.45 Gy carbon ions at postnatal 6 weeks, and then followed by a challenge dose of 5.50 Gy iron ions, and (2) the animals were only total body irradiated with the challenge dose. Closed circles with solid line stand for the groups receiving both the priming dose and the challenge dose at postnatal 6 weeks and postnatal 8 weeks, respectively. Open circles with solid line stand for the groups receiving only the challenge dose at postnatal 8 weeks. The number of CFU-S was measured on the days 11, 12, and 13 after the challenge IR. Data of each experimental point were from 6 to 12 mice. One asterisk (*) stands for statistically significant differences ($P < .05$) between the 2 groups that were compared. Two asterisks (**) indicate statistically significant differences ($P < .01$) between the 2 groups that were compared. C stands for carbon, Ne stands for neon, and Fe stands for iron.

IR was from high-LET carbon particles and the challenge IR was from high LET carbon or neon particles, increased mean number of CFU-S was confirmed. Under AR uninducible condition, when the priming IR was from high LET carbon particles and the challenge IR was from high LET iron particles, no increased mean number of CFU-S was observed.

Discussion

A better understanding of AR and other nontargeted effects is needed to understand to which extent application of low-dose IR might be beneficial to humans.³⁵ To date, investigations

using the AR mouse model (Yonezawa Effect) have obtained many substantial achievements in the study of radiation-induced AR at whole body level. In a series of comprehensive investigations, Yonezawa and colleagues verified the existence of AR under a variety of experimental conditions (ie, doses of priming and challenge IR, intervals between priming and challenge IR, and age and strain of the animals).²³ These efforts helped this AR mouse model to lay a cornerstone for in vivo AR research. Of note, based on the priming dose and the interval between priming and challenge exposures and the timing for delivery of the priming dose, 2 different phenotypes of AR were observed, involving different mechanisms; the first

phenotype was induced 2 weeks after a 0.3 to 0.5 Gy priming IR, which was due to *Trp53*-dependent radioresistance in blood-forming tissues,^{26,36} and the second phenotype was observed 2 months after a 0.05 to 0.1 Gy priming exposure and the AR resulted from the interaction between blood-forming tissue and the central nervous system.^{37,38} As rescue of bone marrow death is the basic criteria for judgment of a successful induction of AR in mice under Yonezawa Effect, studying the recovery of HSCs/HPCs is of critical significance from the point of view of mechanism research, the model for the first phenotype was applied to the present work to validate the HSCs/HPCs measured as endogenous CFU-S under AR inducible and uninducible conditions using combination of low-LET X-rays and high-LET heavy ion IR as priming and challenge IR. It is known that bone marrow, as the site in the body where self-renewal and differentiation of HSCs to mature blood cells mainly occurs, is extremely radiosensitive. Exposure to IR at high doses could devastate bone marrow leading to bone marrow death. In addition, hematopoietic capability is the most critical factor preventing radiation-induced bone marrow death. Even sublethal doses of IR could cause a decrease in hematopoietic cells and a deficit to bone marrow hematopoietic microenvironment. Ionizing radiation-induced decline in total bone marrow hematopoietic cells is accompanied with elevated adipocytes into the marrow cavity, leading consequently to the inhibition of bone marrow microenvironment recovery and hematopoiesis.³⁹ As the number of endogenous CFU-S could reflect both the number of HSCs/HPCs^{32,40} and the environment for hematopoiesis,⁴¹ endogenous CFU-S assay is capable of evaluating the hematopoietic capability.

In this AR mouse model, previous studies showed that successful induction of AR by priming low-LET X-rays or γ -rays was regardless of the dose rate,⁴² and mechanistic study suggested that priming IR-induced decreased p53, Bax, and apoptosis positive cell accumulation in the spleen might favor the recovery of hematopoietic function from challenge IR-induced acute injury, manifesting as stimulated recovery of spleen weight and endogenous CFU-S, contributing to a decrease in bone marrow death.⁴³ Studies on the protective effects induced by low doses of low-LET IR indicated that the underlying mechanisms included enhanced antioxidative capacities, increased cellular DNA repair capacity leading to such as reduced initial DNA damage in AR in mice in vivo,^{44,45} and reduced cell death and mutations in vitro.^{5,46} These induced responses were tightly conserved throughout evolution, being basic responses critical to life.⁴ On the other hand, as high-LET IR from heavy particles induced biological effects qualitatively different from those induced by low-LET IR from photons,⁴⁷ for example, high-LET IR induced more clustered DNA damage and higher rates of residual chromatin breaks,⁴⁸ cellular radiosensitivity correlated with the frequency of residual chromatin breaks,⁴⁹ and the recovery ratio of the potentially lethal damage depended on the quality of IR.⁵⁰ In the present work, increased endogenous CFU-S was observed in the animals under AR inducible conditions, being consistently well with the 30-day survival results: Under AR inducible conditions for

rescue of bone marrow death, AR manifested as significantly increased number of HSCs/HPCs could be induced regardless the priming low dose of IR was from low-LET X-rays or certain high-LET heavy ions such as carbon particles. These results indicate that induction of AR may protect the ability of animals to hinder the decline in the total HSCs/HPCs through possibly inducing radioresistance in the hematopoietic tissue and maintaining the hematopoietic microenvironment. These findings suggest that induction of AR by low-LET and certain high-LET heavy ions may share at least some mechanisms in common. In fact, mechanistic study in vitro in cultured human fibroblasts showed that gene expression profiles following low-LET γ -rays and decays of high-LET like ¹²⁵I shared the majority of genes in common, indicating that both types of IR elicited similar signal transduction pathways,⁵¹ and the extent of DNA clustered damage may not be the major factor modulating gene expression after exposure.⁵² Low doses of low-LET X-rays were effective in reducing chromosomal aberrations and mutation frequency induced by high-LET IR.^{2,53} These findings suggest that the biological defense mechanisms induced by prior low doses from either low-LET IR or high-LET IR may be considered as effective countermeasures, being sufficient enough against the damages caused by subsequent higher challenge doses from either low-LET or high-LET IR. On the other hand, it is noticed that heavy ions with higher atomic number and higher energy (ie, iron particles) failed to induce AR regardless of its use as priming or challenge IR. These findings also suggest that induction of AR may depend on the quality of IR at least to a certain extent. Is there a threshold for the atomic number and higher energy of the heavy ions to induce AR in this mouse model? More questions remain to be answered through further research on especially the underlying molecular mechanisms.

The priming dose used in the present work was higher than 100 mGy which was extensively used in the field of so-called low-dose research. It is known that in the experimental study on AR induction or hormesis, the low doses used in the in vivo systems are often relatively higher than that used in the in vitro systems, and the altitude of the dose seems to be dependent of the biosystems. On the other hand, when thinking about the clinical application of induction of AR or hormesis for the treatment of patients with cancer to protect the normal tissue from being damaged by radiotherapy at high doses, 0.50 Gy could be considered as a low dose. More importantly, application of AR or hormesis should be more practical and acceptable for most of the patients via research and development of medication based on the molecular mechanisms underlying induction of AR or hormesis rather than application via exposure of the patient to priming low dose of radiation.

Taken together, results (Table 2) in the present study showed that under AR inducible conditions, regardless of the quality of IR (ie, low LET and high LET, photons and particles) for the combination of the priming dose and the challenge dose, the priming IR could induce an increased number of HSCs/HPCs as measured by the number of endogenous CFU-S, which may contribute to the rescue of bone marrow death.

Table 2. Measurement of CFU-S under AR Inducible and Uninducible Conditions in Mice.

AR Inducible Conditions	Dose, Gy		Consistency of CFU-S Increase with AR Induction Conditions
	Priming IR	Challenge IR	
Yes	X-rays (0.50)	X-rays (5.00)	Yes
Yes	X-rays (0.50)	C (5.00)	Yes
No	X-rays (0.50)	Fe (5.50)	Yes
Yes	C (0.45)	X-rays (4.75)	Yes
No	Fe (0.45)	X-rays (4.75)	Yes
Yes	C (0.45)	C (5.25)	Yes
Yes	C (0.45)	Ne (5.00)	Yes
No	C (0.45)	Fe (5.50)	Yes

Abbreviations: AR, adaptive response; CFU-S, colony forming units-spleen; IR, ionizing radiation.

Results indicated the significance and possible application of AR to the reduction in acute radiation syndrome induced by high dose from either low- or high-LET IR. These findings bring new knowledge to the characterization of the Yonezawa Effect by providing a new insight into the mechanistic study on the hematopoietic system in the AR mouse model *in vivo*.

Acknowledgments

The authors thank all the staff of Experiment Support Group, Accelerator Division, and Accelerator Engineering Corporation, for performing heavy-ion irradiations. The expert technical assistance and administrative support of Ms Mikiko Nakajima, Ms Nobuko Tsutsumi, Ms Yasuko Morimoto, Ms Satoko Idohara, Ms Ichika Kishi, Ms Kaori Tateno, Mr Tatsuo Hayao, Dr Toshiaki Kokubo, Ms Maki Asano, and Ms Hiromi Arai is gratefully acknowledged. The authors also thank Dr Yi Shang for her critical and constructive comments on the experimental design, performance, data analysis, and manuscript preparation. We are also deeply grateful to Dr Takeshi Murakami for his continual support that made this study possible. This article is dedicated to late Dr Takeo Ohnishi, professor emeritus, Nara Medical University School of Medicine, for his constructive comments and continual encouragement throughout the study. Thanks are also due to the anonymous peer reviewers for providing the constructive comments that strengthened the presentation of this work.

Authors' Note

Bing Wang and Kaoru Tanaka contributed equally to this work.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was partially supported by MEXT Grant-in-Aid for Scientific Research on Innovative Areas "Living in Space" (Grant Numbers: 15H05935, 15K21745), JSPS KAKENHI 25340041, and 3 Research Project Grants (19B258, 22B258, and 14J286) with Heavy Ions at NIRS-HIMAC, Japan.

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