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Low-Dose-Rate Irradiation for 1 Hour Induces Protection Against Lethal Radiation Doses but Does Not Affect Life Span of DBA/2 Mice

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Abstract

Prior findings showed that serum from DBA/2 mice that had been given whole-body irradiation for 1 hour at a low dose rate (LDR) of 30 cGy/h induced protection against radiation in reporter cells by a mechanism depending on transforming growth factor β3 and inducible nitric oxide synthase activity. In the present study, the effect of the 1 hour of LDR irradiation on the response of the preirradiated mice to a subsequent lethal dose and on the life span is examined. These DBA/2 mice were prime irradiated for 1 hour at 30 cGy/h. Two experiments with 9 and 9.5 Gy challenge doses given 6 weeks after priming showed increased survival in primed mice compared to unprimed mice followed up to 225 and 81 days after challenge irradiation, respectively. There was no overall significant difference in life span between primed and unprimed mice when no challenge irradiation was given. The males seemed to have a slight increase in lifespan after priming while the opposite was seen for the females.

Keywords

TGF-β3, life span, adaptive effect, LD_{50}, priming irradiation

Introduction

High acute doses of ionizing radiation are known to be harmful to living organisms. However, at low doses and dose rates, radioadaptive responses have been observed in vitro and in vivo using various end points, such as cell lethality, chromosomal aberrations, mutation induction, radiosensitivity, and DNA repair.[1-4] Adaptation in vitro is most efficiently induced by doses of 1 to 50 cGy at dose rates from 0.6 to 60 Gy/h[4], with challenge doses in the range of 0.5 to 2 Gy.[5] The protective effect has been reported to last for up to 3 cell generations following acute priming irradiation in lymphocytes.[6]

In vivo an adaptive effect of an acute priming dose of 2.5 to 50 cGy has been seen in measurements of 30-day survival of ICR mice after a challenge dose of 8 Gy.[7] Other types of adaptive or improved responses have been measured in vivo after low-dose-rate (LDR) priming irradiation; long-term LDR preirradiation has been shown to reduce the incidence of chemically and radiation-induced tumors.[8,9] Several studies have shown prolongation of life span after long-term LDR irradiation for mice not given a challenge dose.[10-14] One should, however, notice that there are also studies that contrast to these findings. For example, a study by Shin et al showed that the average life span was significantly lower than unirradiated controls in AKR/J mice irradiated with a total of 4.5 Gy at either LDR (0.7 mGy/h) or high dose rate (48 Gy/h). However, the incidence of thymic lymphoma was lower in LDR irradiated mice (ie, 10% lower than control; 20% lower than high dose rate).[15] Significant life shortening was also observed by Thomson et al in male B6CF1 mice (a mouse strain in which mortality is affected by the incidence of various cancers) exposed to 0.8 mGy/h for 23 weeks or 7.5 mGy/h for 59 weeks[16] and by Tanaka et al using the same mouse strain irradiated for approximately 400 consecutive days

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(starting week 8) with a dose rate of 21 mGy/d. Tanaka et al however found no effect on life span for dose rates of 1.1 or 0.05 mGy/d.[17]

Other studies found no effect on life span by chronic irradiation at dose rates of 1 and 2 mGy/h,[18] 4 mGy/h and 50 cGy/h,[19] or 10 cGy/year (0.011 mGy/h).[20]

In the present study, we have given DBA/2 mice an LDR whole-body priming dose of 30 cGy given over 1 hour (30 cGy/h). The priming irradiation used in the present study was chosen on the basis of our earlier studies partly performed on in vitro cells and partly on DBA/2 mice. It was essential that the priming was given as an LDR irradiation. The background for that was our earlier finding that 30 cGy given acutely to human T-47D cells induced cell death in about 20% of the cells (hyperradiosensitivity), whereas the same dose given protracted during 1 hour (30 cGy/h) did not induce significant cell killing.[21,22] Our data furthermore indicated that transforming growth factor β3 (TGF-β3) was induced by this priming irradiation and that addition of active TGF-β3 to the cells before irradiation could even protect T-47D cells against a larger challenge dose given after the priming dose.[23]

In the follow-up to these in vitro studies, we used the T-47D cells as in vitro reporter cells to test whether these mechanisms could be initiated by priming irradiation of an animal: We gave DBA/2 mice a whole-body priming dose of 30 cGy at the LDR of 30 cGy/h and subsequently harvested serum, which was transferred to reporter T-47D cells cultured in vitro. The response of the reporter cells to a challenge dose in the presence of the mouse serum was then tested. It turned out that serum from unprimed mice had no effect on the radiosensitivity of the reporter cells, while serum from primed mice increased radioresistance of the reporter cells to all doses given (up to 5 Gy was tested). The effect of the serum was seen to depend on TGF-β3 during reporter cell exposure and on inducible nitric oxide synthase (iNOS) activity in the mice before the serum was harvested.[24]

In the light of these results, we wondered if the mechanisms activated in the mouse body by the priming dose could not only protect reporter cells against a challenge dose but perhaps even improve the mouse survival after a whole-body, lethal challenge dose (LD_{50}). We conclude that whole-body preirradiation with 30 cGy/h protects DBA/2 mice against a subsequent single whole-body LD_{50} (9-9.5 Gy) challenge dose, but it does not affect the life span of mice not given a challenge dose.

Materials and Methods

Mouse Model and Irradiation

Inbred nonspecific pathogen-free DBA/2 mice[25] were used. After sex segregation at weaning, they were kept 8 per cage and fed mouse pellets and water ad libitum. The animal room, the animal care, and the experimental use of the animals were in accordance with the Slovak Ethical rules. The mice were maintained, cared, and used in accordance with institutional guidelines under the approved protocols by the ethical committee and the Slovak Veterinary Office.

All mice were bred locally in a nonbarrier unit. Mice were collected for each experiment as they were bred with the result that the age at the time of the priming irradiation could vary from 2 to 5 months.

Animals were placed in a circular pen with wedge-shaped individual rooms, giving each animal an identical exposure to the radiation. A ^{60}Co-source (Theratron Elite 100; Best Theratronics, Canada) was used. The priming dose rate was obtained with total cerrobend filtration in solid Poly(methyl methacrylate) [PMMA] phantom by optimal source to skin distance (SSD) (−217 cm) for a dose rate of the 30 cGy/h with field size 35×35 cm^2. The time for application of 30 cGy was in the interval 59.6 to 60.3 minutes. The treatment parameters were corrected at each application to ensure that the mice received the demanded dose rate. The dose rate was controlled by an ionization chamber (FC-65G; Wellhöfer, Germany) calibrated by standard laboratory IAEA Seibersdorf.

Two different types of experiments were conducted. In the first 2 experiments (Figures 1 and 2), primed and nonprimed mice were exposed to a challenge dose of 9 or 9.5 Gy. Sixty-four mice (16 females and 16 males were primed and the same number nonprimed) were used in the experiment shown in Figure 1, and 36 mice (12 females and 8 males were primed and 8 of each nonprimed) were used in the experiment shown in Figure 2. The dose rate used for challenge doses of 9 and 9.5 Gy was 55.8 Gy/h, and the time between priming and challenge irradiation was about 6 weeks. In order to minimize the possible effects of age-related differences in responses to the priming irradiation, the mice for priming and control groups were age matched.

In the second type of experiment (Figure 3), life span was recorded of mice that were not challenge irradiated. Ninety-nine mice (47 females and 52 males) were primed with 30 cGy for 1 hour, and 159 (47 females and 112 males) were used as controls. The mice were monitored regularly every second day by estimation of their weight. In the period of frequent death because of irradiation, they were checked every day. Mice with life expectancy of few hours (moribund) were euthanized by an overdose of anesthetics. Autopsy was seldom performed, only when the course of death was not clear. Histopathology was not performed.

The mice in the life span study (Figure 3) died of old age after turning gray. In the mice irradiated with the challenge doses (Figures 1 and 2), a gradual loss in body weight was observed at first. At autopsy, intestinal bleeding was noticed. Diarrhea or hair loss was not observed, but some of the mice turned gray.

Statistical Analyses

Statistical analyses were performed using Student t test and Mantel-Cox (log rank) test to compare life spans of LDR primed mice versus unprimed mice (Figure 3). For the data where a challenge radiation dose of either 9 or 9.5 Gy was given with or without previous priming (Figures 1 and 2), Mantel-Cox (log rank) test was used. P values $<.05$ were considered significant.

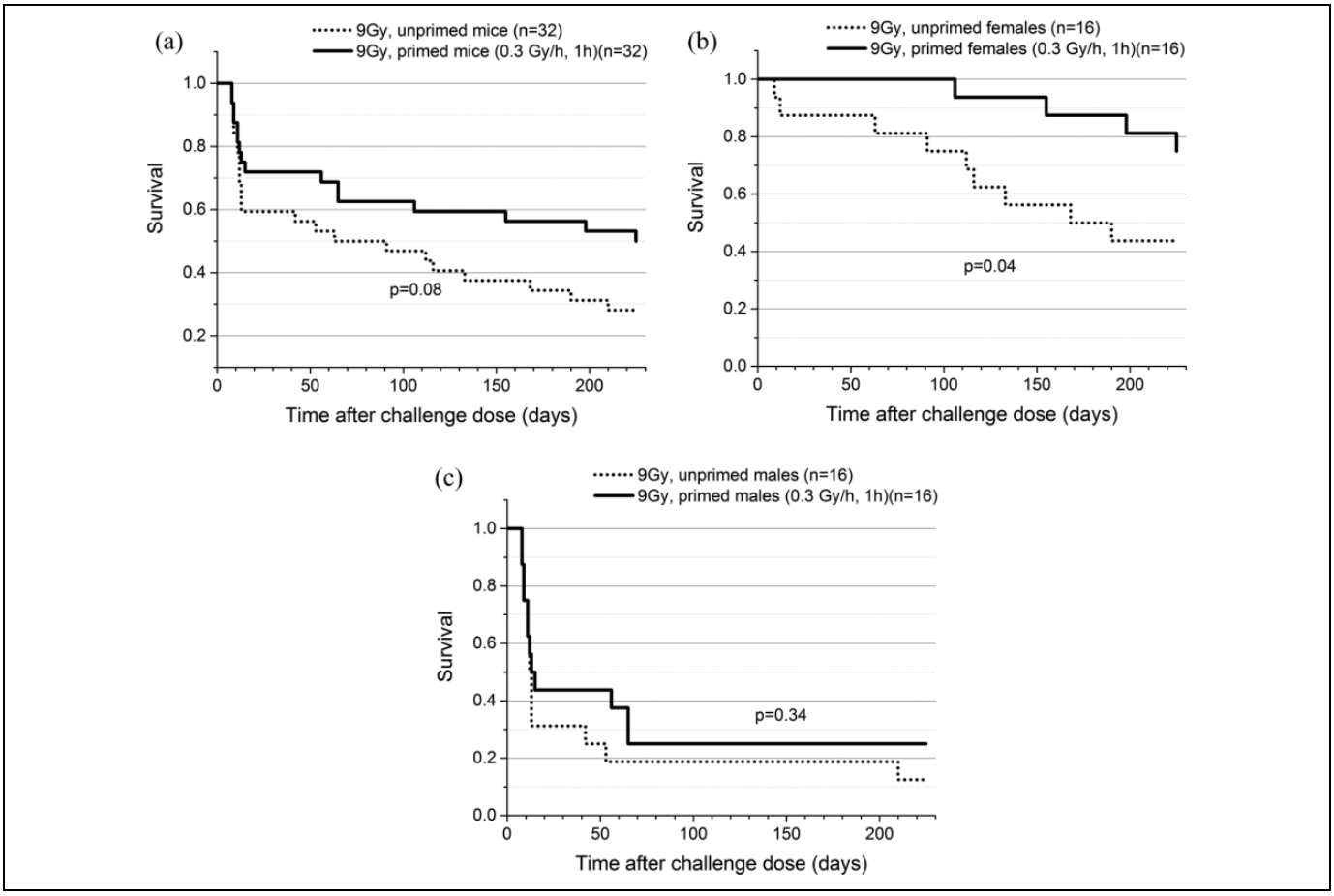

Figure 1. Life span after a challenge dose of 9 Gy. Sixteen males and 16 females were first given a 1-hour priming irradiation at 30 cGy/h. Six weeks later, they were irradiated with a high-dose-rate challenge dose of 9 Gy. Sixteen males and 16 females (age matched to the primed mice) were challenge irradiated without a priming dose. A, Males and females pooled. B, Females. C, Males.

Results

Figure 1 shows the fraction of mice surviving as a function of the time after a 9 Gy whole-body challenge dose. Separate curves are plotted for animals given a prechallenge priming dose of 0.3 Gy with an LDR of 0.3 Gy/h 6 weeks before the challenge dose and for animals given the challenge dose without any priming irradiation. The pooled data (Figure 1A) indicate that the median survival time increased from 90 days for unprimed mice to over 230 days for primed mice. This difference is not statistically significant by a Mantel-Cox (log rank) test ($P = .08$). There is, however, a marked difference between the genders: A significant increase in life span in primed compared to unprimed mice was seen in females (Figure 1B). The data on males however indicated only a slight increase in the life span for the primed compared to the unprimed animals, a difference not statistically significant (Figure 1C).

The experiment was repeated with a slightly higher challenge dose of 9.5 Gy but with the same priming irradiation as was used in the first experiment (Figure 2). Unfortunately, the follow-up time in the second experiment had to be shortened to just 81 days due to technical reorganization of the animal house. In the cage, holding female mice given a priming dose, no deaths were observed during these 81 days of follow-up. In this second experiment, however, there was a significantly increased life span for primed versus unprimed mice when data for both genders were pooled together ($P = .001$). The statistical significance was also clear for both genders separately (Figure 2B, $P = .02$ for females and Figure 2C, $P = .001$ for males).

It is not known whether the protective effect of priming irradiation on a subsequent acute challenge irradiation has to do with increased repair capacity within individual cells or perhaps some systemic effect like, for example, stimulation of the immune system protecting the mice from detrimental effects from the microflora. Such detrimental effects could also be of importance for the normal life span of the animals, that is, animals not exposed to any challenge dose, experiencing senescence before death. We therefore wanted to test whether the LDR priming had any protective or detrimental long-term effects in mice not given a challenge dose.

A test of this kind, without a challenge dose, needs a high number of animals and a very long follow-up time. In our test, the life span of 99 LDR primed mice was compared to that of

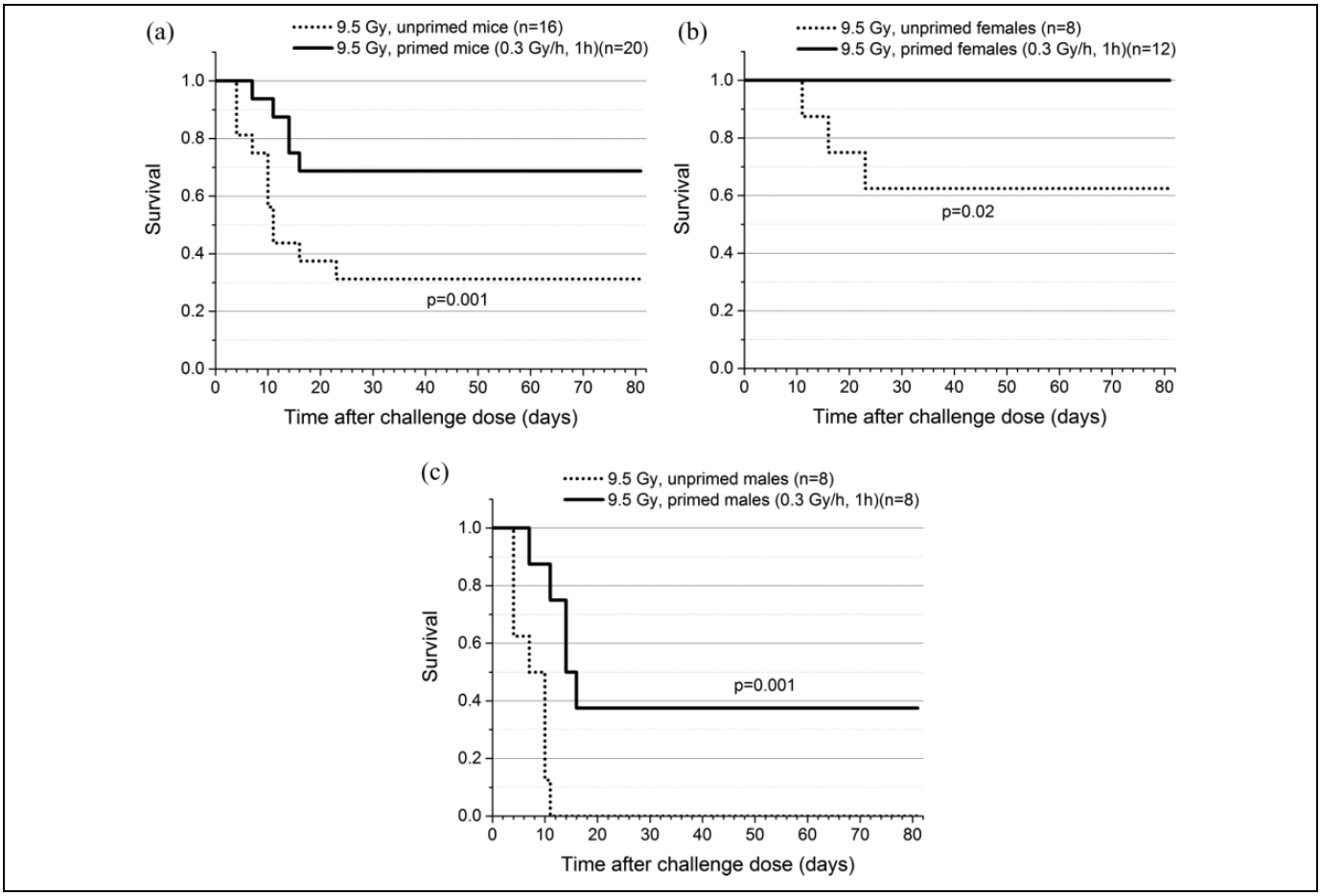

Figure 2. Life span after a challenge dose of 9.5 Gy. Eight males and 12 females were first given a 1-hour priming irradiation at 30 cGy/h, 6 weeks before the high-dose-rate challenge dose of 9.5 Gy. Eight males and 8 females (age matched to the primed mice) were challenge irradiated without a priming dose. A, Males and females pooled. B, Females. C, Males.

159 unprimed controls with a follow-up of 1200 days, which roughly covers the normal life duration for a mouse (Figure 3). The data showed no significant difference between the life spans of primed compared to unprimed mice. Mantel-Cox test gave *P* values of .66 for females, .11 for males, and .23 for pooled data from both genders. There was, however, a higher mortality rate in the earlier time intervals for the primed females than any of the other populations (see Supplementary Figure S1). In order to see if there could be specific effects related to a certain cage, we replotted the data from Figure 3 also with individual time of death for each animal per cage in Supplementary Figure S2. Apart from an observation of 3 very long-lived unprimed females living in the same cage, there was no indication of special effects related to single cages.

The mice were bred locally and collected for priming, which could only be done at certain times. The age of the mice at the time of priming could therefore vary. The effect of age at the time of priming irradiation on the mean life span is plotted in Supplementary Figure S3. Linear fittings gave for primed females a slope of 1.5 ± 0.9, and for males 2.1 ± 4.4. Thus, a correlation cannot be ruled out for the females who also have the largest variation in age at priming.

Discussion

In the present study, the time between priming and challenge irradiation was 6 weeks. This was just a practical choice. In our previous studies, we found that radioprotective effects were seen in reporter cells exposed to serum from primed mice even when the serum was harvested up to 15 months after priming irradiation.[24] In both cases, involvement of TGF-β3 and iNOS activity was demonstrated,[23,24,26] which makes it tempting to speculate that TGF-β3 and iNOS activity may play a role in the adaptive effect observed in Figures 1 and 2. However, this remains to be further investigated.

The LD_{50} for mice is higher than for humans ($\sim$4.5 Gy).[27] It can therefore be debated whether the priming dose/dose rate should be adjusted for humans as compared to mice. Our earlier in vitro studies showed that LDRs of 30 cGy/h as used in the present study (6 cGy/h was the lowest tested) induced the same effect as long as the irradiation time was as prolonged as 1 hour. The protective effect vanished if the priming irradiation time was reduced to 15 minutes.[26] In the experiments with reporter cells exposed to serum from irradiated mice, the effect was also seen for 3 cGy/h.[24] The effect seems thus to be dose

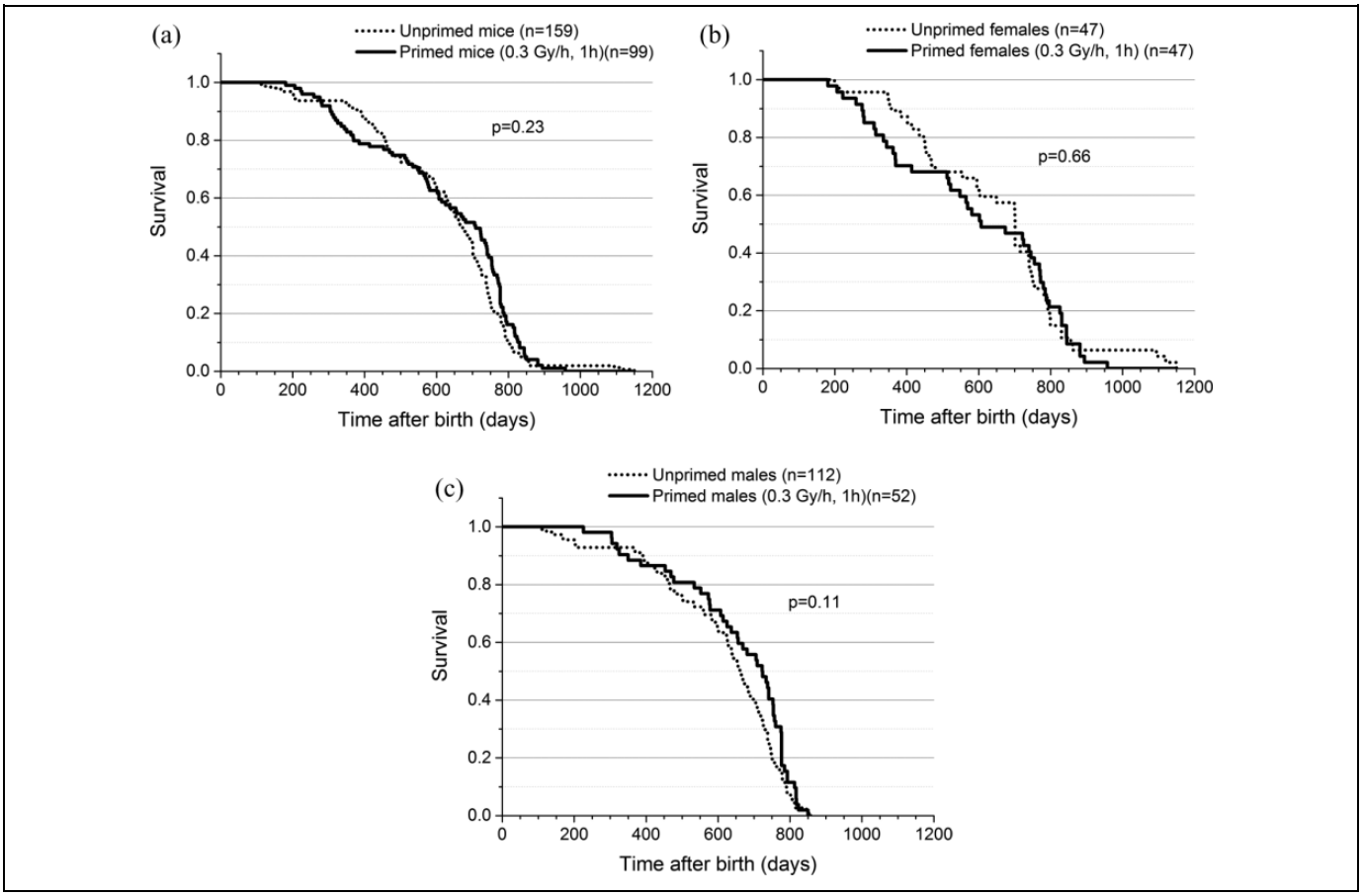

Figure 3. Life span in mice exposed to 1-hour priming irradiation at 30 cGy/h compared to unprimed mice. The mice were primed at ages 2 to 5 months. The effect of age at the time of priming on life span is negligible (shown in Supplement Figure 3). A, Males and Females pooled. B, Females. C, Males.

independent as long as the dose rate is within a certain window and the irradiation time is long enough. Our data confirm that the used priming dose induced similar mechanisms in mice as in human cell cultures.

In light of the improved survival for the primed compared to the unprimed animals after a lethal challenge dose, it was interesting to evaluate whether the priming dose used had an impact on the life span of mice not exposed to a lethal irradiation dose. However, no significant differences were seen regarding life span in the primed versus unprimed mice (Figure 3A). There seems to be a slight tendency of longer life span in primed males compared to controls (Figure 3C), whereas the opposite trend appears for females (Figure 3B). The primed males also seemed to live longer (not significant) than primed females ($P = .16$). In the females, the data show some early deaths in the primed mice and 3 very long-lived controls. The early deaths in 2 cages coincided with early age at the time of priming (Supplementary Figure S3). Interestingly, the 3 long-lived female controls lived in the same cage, and the early deaths in the primed females also seemed to appear in certain cages (Supplementary Figure S2). Thus, it is possible that the life span in the females was influenced by unknown interactions between mice housed in the same cage and a larger variation in age at priming than in the males, in particular, 3 cages primed before 37 days of age.

Altogether, we conclude that with the size of the present study, it is not possible to see any significant change in life span after 1 hour of γ-irradiation at 30 cGy/h in otherwise unirradiated mice. The variations are more likely related to other factors in connection with keeping mice in cages of 8 in a nonbarrier unit and variations in age at the time of priming.

The International Commission on Radiological Protection suggests a threshold dose for damaging effects of 10 cGy.[28] Our previous and present data indicate that with an LDR of 30 Gy/h, even a dose of 30 cGy to the mice results in a response characterized by no change in life span. This is in line with a study by Thomson et al in which B6CF1 mice were irradiated with cobalt-60 γ-rays. The dose rates were adjusted to give 22.5, 45, or 90 cGy in 20 minutes, corresponding to 67.5, 135, and 270 cGy/h. The 2 lowest doses/dose rates are comparable to the irradiation used in the present study, and no significant change in life span was observed after these.[29]

In conclusion, DBA/2 mice given an LD_{50} of 9 or 9.5 Gy experienced significantly improved long-term survival if they had been given a 1-hour whole-body priming γ-irradiation with 30 cGy before the lethal challenge dose as compared to mice

without priming irradiation. The protection seems to be long lasting as the lethal challenge radiation doses were given 6 weeks after priming. No significant change in life span was observed due to priming in the mice not given a challenge dose.

Declaration of Conflicting Interests

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Supplemental Material

The online supplemental figures are available at http://dos.sagepub.com/supplemental.

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