Different Radiation Susceptibility among Five Strains of Mice Detected by a Skin Reaction

MAYUMI IWAKAWA1*, SHUHEI NODA1, TOSHIE OHTA1, CHISA OHIRA1, RYONFA LEE1, MIYAKO GOTO1, MIYUKI WAKABAYASHI1, YOSHIFUMI MATSUI1, YOSHINOBU HARADA1 and TAKASHI IMAI1

Radiosensitivity/Strain difference/Skin reaction

Published reports about skin reactions to radiotherapy, especially among breast-cancer patients, suggest that there are interindividual differences in the normal tissue response, and genetic factors are thought to be involved in this variation. An analysis of murine strain differences may reveal the mechanism of genetic factors in the extent of normal tissue damage from irradiation for several endpoints. The variation in the radiation susceptibility was observed when the skin of mice from strains A/J, C3H/HeMs, C57BL/6J, C.B.17/ICR-scid and C3H-scid was irradiated with a single dose ranging from 10 to 60 Gy, using Cs-137 gamma rays. The active skin reaction of A/J mice lasted for months. C3H/HeMs mice showed dose-dependent skin damage, and consequently recovered to a state of mild damage within 40 days after local irradiation. The time course of the response in C57BL/6J mice was shorter than in A/J mice. The 2 strains of scid mice exhibited severe damage after irradiation at any dose from 20 to 50 Gy, and did not show any dose dependency. The variation between murine strains in macroscopic and histopathological changes in skin during the progression and resolution of damage caused by irradiation suggests an inter-strain variation in the expression of genes involved in injury, apoptosis, repair, and remodeling.

INTRODUCTION

During the time that orthovoltage radiotherapy was in use, the skin reaction was a critical complication that was sometimes a dose-limiting factor. Even today, erythema and desquamation occur as a consequence of the radiotherapy of some tumors. Published reports of skin reactions, especially among breast-cancer patients, suggest that there are interindividual differences in the normal tissue response to radiotherapy, and genetic factors are thought to be involved in this variation1-2. The analysis of murine strain differences, together with breeding studies, has demonstrated that there are genetic factors in the extent of normal tissue damage from irradiation for several clonogenic endpoints3-4. Weil et al. reported that the difference in the susceptibility to radiation-induced jejunal crypt cell apoptosis between C57BL/6J and C3H/Kam mice is a heritable trait that is likely to be controlled by a set of genes distinct from the set that controls the thymocyte apoptosis levels in the thymus5-6. Franko et al. reported on studies of radiation-induced lung injury in several murine strains7-8. Studies have revealed differences in the sensitivity of lungs and the timing of the development of injury between strains8,10. In this study, differences in the skin reaction after a graded single dose among 5 strains of mice were examined, as part of an attempt to identify genes involved in individual radiosusceptibility.

MATERIALS AND METHODS

Mice

Female mice of the inbred strains A/J, C57BL/6J, C3H/HeMs, C.B.17/ICR-scid and C3Smn.CB17-Prkdcscid/J (common name: C3H-scid) were used. The mice were obtained and maintained from specific-pathogen-free mice colonies at the National Institute of Radiological Sciences. The mice were 12 weeks old at the time of irradiation. A maximum of 6 mice were kept in each cage. To examine the macroscopic skin reaction, 6 mice from each strain received one of the doses of irradiation used. To examine skin histopathology, 3 mice from each strain were irradiated with a single dose of 50 Gy, and one mouse from each strain was sacrificed by spinal dislocation at 10 days, 20 days and 30 days after irradiation. A total of 217 mice were used in this study. Our methods were reviewed and approved by the NIRS Institutional Animal Care and Use Committee, as protocol number 13-1073.

*Corresponding author: Phone: +81-43-206-3062, Fax: +81-43-206-6267, E-mail: mayumii@nirs.go.jp
1Frontier Research Center, National Institute of Radiological Sciences, 4-9-1 Anagawa, Inage-ku, Chiba-shi, Chiba-ken 263-8555, Japan.
Irradiation

The skin of the right hind leg was chemically depilated 5 days before irradiation. Local irradiation was performed using a beam of Cs-137 gamma rays at an FSD of 21 cm with a dose rate of 1.4 Gy/min. A toroidal magnetic field with a 30-mm rim was used to collimate the vertical beam. Mice were anesthetized with pentobarbital at a dose of 50 mg per kg body weight before irradiation. Adhesive tape was used to immobilize the mice on a Lucite plate and position their right-hind legs. Irradiation was performed at graded single doses ranging from 20 to 60 Gy for 3 strains, or 10 to 50 Gy for 2 strains of scid mice, with 6 mice of each strain left unirradiated to serve as controls.

Skin Reaction

The acute skin reaction following graded single doses of gamma rays was scored every other day for 150 days after irradiation, using the arbitrary scale shown in Table 1. The scoring system consisted of 10 degrees, ranging from 0.5 to 3.5 (Table 1).

Histopathology

A piece of irradiated skin with an area of 2 cm² was examined for histopathological changes. This skin was fixed in 10% neutralized formalin and embedded in paraffin. Sections were cut at a width of 3 microns and stained with hematoxylin and eosin (H&E).

RESULTS

Radiosusceptibility of skin

The 5 different strains showed different degrees of susceptibility to single-dose skin irradiation. All 5 strains showed skin reactions approximately 8 days after irradiation; i.e., there were no differences in the latent periods of damage among the 5 strains. The skin reaction of A/J mice lasted for months (Fig. 1A). All irradiated C3H/HeMs mice recovered to a score of 0.5 after day 40 (Fig. 1B). C57BL/6J and A/J mice had similar time courses of response (Fig. 1C). Two strains of scid mice (C.B.17/Icr-scid and C3H-scid), which are known to be mutant in Prkdc, the gene encoding DNA-PKcs, exhibited severe skin damage from radiation. Also, systemic weakness was observed in C3H-scid mice after local irradiation; because C3H-scid mice that were irradiated at a single dose of more than 20 Gy died within 60 days, the data of the C.B.17/Icr-scid mice demonstrate Fig. 1D. Figure 2(A) shows the skin reactions of 4 murine strains after local irradiation with a single dose of 50 Gy. There were differences in the time course of recovery among these 4 murine strains. The peak skin reaction score at each dose group was plotted for each strain (Fig. 3). A dose-response analysis yielded regression equations, and a regression line was obtained; a dose-response analysis yielded regression formulas for the following 3 strains: C3H/HeMs, y = -0.5195 + 0.05417x (R² = 0.9894); A/J, y = -0.1707 + 0.05074x (R² = 0.9741); C57BL/6J, y = -0.1078 + 0.04938x (R² = 0.9346). The slopes of those 3 linear equations were nearly identical. The 2 strains of scid mice did not exhibit any dose dependency within the dose range between 20 and 50 Gy. When we compared the time course of the skin reaction after irradiation at a dose of 30 Gy, the following order of radiosensitivity to skin irradiation was obtained: C3H scid (most sensitive) > C.B.17/Icr-scid > C57BL/6J > A/J > C3H/HeMs (least sensitive) (Fig. 2B).

Histopathological findings

A macroscopic observation of the skin reaction after irradiation revealed that C3H/HeMs mice showed rapid healing, whereas C57BL/6J and A/J demonstrated long-standing erosion. To precisely investigate the process of injury and repair, histological changes after skin irradiation were observed in 3 strains: C3H/HeMs, C57BL/6J and A/J. The hind legs of a group of C3H/HeMs, C57BL/6J and A/J mice were exposed to 50 Gy of irradiation. Animals were sacrificed 10, 20, or 40 days after irradiation. The unirradiated murine skin had a few layers of epidermis and loose connective tissue (Fig. 4). Ten days after irradiation, in A/J and C57BL/6J mice, the desquamation or epidermal ulceration was observed (Fig. 5A, C). In C3H/HeMs mice, with the erosion and formation of an ulcer base, rapid epithelialization and moderate acanthosis were detected in C3H/HeMs mice (Fig. 5B). At 40 days the epidermal damage in C3H/HeMs mice was on the average less severe (no ulcer base and moderate acanthosis) than that in A/J or C57BL/6J mice (Fig. 6). In A/J mice, more severe damage, such as the total loss of epidermis or a wide ulcer base or severe infiltration of inflammatory cells, was observed (Fig. 6A1, 6A2). In C57BL/6J mice, a thicker epidermis was noticed with hyperkeratosis (Fig. 6C).

DISCUSSION

Many studies of the inter-strain differences in the responses of mice to irradiation have been conducted in order to clarify the mechanisms of radiotiosensitivity. In particular, there have been many studies involving murine in situ clonogenic assays, includ-
Gasinska et al found differences in the radiosensitivity of spermatogenesis between the 4 inbred strains, such as KE, KP, CBA/KW and C57/KW. In contrast, use of repeatable and quantitative assays that depend on functional endpoints has been declining. In the present study, the skin reaction as a functional endpoint, which is applicable to clinical investigation, was compared among 5 mouse strains, including 2 strains of scid mice.

Scid mice are known to be radiosensitive, with a susceptibility to DNA double-strand breaks, due to a mutation in the Prkdc gene. We were unable to fully study the skin reaction in C3H-scid mice because of their high mortality, even after local irradiation. The skin reaction data of C.B.17/Icr-scid mice indicated that damage to target cells (skin) by irradiation was not dose-dependent. Okayasu et al identified 2 BALB/c strain-specific polymorphisms in the coding region of the Prkdc gene, which is known to be involved in the post-irradiation signal transduction and repair of DNA double-stranded breaks. They also observed a significant difference in the repair kinetics between cells from scid and BALB/c strains, and found that the repair of DNA double-strand breaks was very efficient in A/J, C3H/HeMs and C57BL/6J strains, with no significant difference between them. They concluded that almost all in vivo and cellular measures of radiosensitivity show quantitative genetic traits influenced by the specific distribution of variant germ-line sensitivity/resistance alleles between different mouse strains.

In the present study, we observed a strain-dependent variation in the patterns of progression/regression after skin damage...
caused by irradiation. The onset of a skin reaction was sudden, and there was no significant difference in the latency between the murine strains or the irradiation doses. We speculate that there are 3 likely explanations for this. First, a dose of 20 Gy to 60 Gy may be too high to allow the observation of slight changes or moderate reactions. Second, the anatomical characteristics of murine skin, i.e. thin layers of epidermis and much loose connective tissue, different from human skin, could not present the protective skin mechanism from insult with a dose-dependent manner elegantly. The anatomical difference in the skin between mice and humans might make the mouse an imperfect model for the skin response in humans. Third, if we perform an experiment using different endpoints or using a fractionation treatment with a small fraction dose, we might observe a dose-dependent latency, which is often observed clinically in cases of skin contraction and radiation responses of the kidneys, ureters, bladder, lungs, spinal cord and myocardium. Instead of latency for onset, the peak reaction showed a dose dependency in A/J, C3H/HeMs and C57BL/6J mice (Fig. 2), although these 3 strains exhibited different processes of repair and healing. Using a method similar to that used in previous wound-healing studies, we investigated histological changes in the skin of the leg after irradiation (Figs. 4, 5, and 6). With macroscopic and microscopic examinations of skin specimens, we observed differences in aspects of step-wise skin reactions, such as epidermal cell loss, hyperplastic epidermis, acanthosis and rapid re-epithelization among A/J, C3H/HeMs and C57BL/6J mice. Rapid re-epithelization occurred at the hyperplastic epidermis in C3H/HeMs mice in the early stage after irradiation. There were some marked discrepancies between the data from macroscopic examinations and microscopic histopathological examinations. Macroscopically, C3H/HeMs mice appeared to be the most radioresistant; they had the lowest skin reaction scores after irradiation. However, a microscopic examination showed that C3H/HeMs mice had the most hyperplastic epidermis as acute response, and that they experienced less influx of inflammatory cells. Dynamic repair and remodeling via the biological response, including the recognition of cell loss or insult to target cells, transduction of this signal, and repair mechanisms including rapid clearance of damaged cells, may reduce the damage to target tissue. It has been reported that the irradiation of murine lung, intestine, bladder or liver is associated with increased TGF-beta expression. Furthermore, skin damage caused by irradiation has inflammatory and fibrotic sequelae related not only to TGF-beta and smad 2 and 3, but also to collagen type I and III. Flanders et al reported that mice null for Smad3 exhibit accelerated healing of cutaneous injury induced by ionizing radiation. Skin from Smad3-/mice exhibited significantly less epidermal acanthosis and der-
Mal influx of mast cells, macrophages and neutrophils\textsuperscript{18}). The obvious differences observed between murine strains in the present study may be due to genomic differences in the genes which are recognized to mediate the process of inflammation and biochemical sequelae, such as TGF-beta and related genes. We are currently conducting a further analysis using microarray assays of samples of irradiated murine skin, to investigate genes that may be involved in individual radiosensitivity. In 21 centuries, intensity modulation radiation therapy has been reported to be a promising new form of radiotherapy\textsuperscript{21}). Even with such advances in technology, a study of patient factors contributing to normal tissue response has important implications.

Fig. 4. H&E-stained sections of (A) A/J and (B) C3H/HeMs, (C) C57BL/6J mouse unirradiated skin, showing several layers of epidermis and loose connective tissue (∼200).

Fig. 5. H&E-stained sections of (A) A/J and (B) C3H/HeMs, (C) C57BL/6J mouse skin 10 days after exposure to gamma rays. Ten days after irradiation, in A/J and C57BL/6J mice, desquamation or epidermal ulceration was observed (A, C). In C3H/HeMs mice, with erosion and formation of ulcer base, rapid re-epithelialization and moderate acanthosis were detected in C3H/HeMs mice (B) (∼200).
for radiotherapy. The ultimate aim of our research is to clarify the mechanisms of heterogeneity in responses to ionizing radiation arising from a genetic variation between individual humans. The inter-strain differences in the murine radiation susceptibility observed in the present study strongly support the hypothesis that genetic factors are important determinants in responses against irradiation\textsuperscript{22}).

**CONCLUSION**

We observed inter-strain differences in radiation susceptibility among 5 murine strains, including 2 strains of scid mice, using the functional endpoint of the skin reaction as an acute response. The variation which we observed between murine strains in macroscopic and histopathological changes in skin during the progression and resolution of damage caused by irradiation suggest an inter-strain variation in the expression of genes involved in injury, repair and remodeling.

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**Fig. 6.** H&E-stained sections of (A) A/J and (B) C3H/HeMs, (C) C57BL/6J mouse skin 40 days after exposure to gamma rays. At 40 days the epidermal damage in C3H/HeMs mice was on average less severe (no ulcer base and moderate acanthosis) than that in A/J or C57BL/6J mice. In A/J mice, more severe damage, such as total loss of epidermis or wide ulcer base or severe infiltration of inflammatory cells, was observed (A1, A2). In C57BL/6J mice, thicker epidermis was noticed with hyperkeratosis (C). (×200).
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