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Effects of ionizing radiation on differentiation of murine bone marrow cells into mast cells

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ABSTRACT

Mast cells, immune effector cells produced from bone marrow cells, play a major role in immunoglobulin E–mediated allergic responses. Ionizing radiation affects the functions of mast cells, which are involved in radiation-induced tissue damage. However, whether ionizing radiation affects the differential induction of mast cells is unknown. Here we investigated whether bone marrow cells of X-irradiated mice differentiated into mast cells. To induce mast cells, bone marrow cells from X-irradiated and unirradiated mice were cultured in the presence of cytokines required for mast cell induction. Although irradiation at 0.5 Gy and 2 Gy decreased the number of bone marrow cells 1 day post-irradiation, the cultured bone marrow cells of X-irradiated and unirradiated mice both expressed mast cell–related cell-surface antigens. However, the percentage of mast cells in the irradiated group was lower than in the unirradiated group. Similar decreases in the percentage of mast cells induced in the presence of X-irradiation were observed 10 days post irradiation, although the number of bone marrow cells in irradiated mice had recovered by this time. Analysis of mast cell function showed that degranulation of mast cells after immunoglobulin E–mediated allergen recognition was significantly higher in the X-irradiated group compared with in the unirradiated group. In conclusion, bone marrow cells of X-irradiated mice differentiated into mast cells, but ionizing radiation affected the differentiation efficiency and function of mast cells.

KEYWORDS: allergic responses, ionizing radiation, differential induction, mast cells

INTRODUCTION

Mast cells are an immune cell type derived from hematopoietic stem/progenitor cells and are systemically present [1]. Mast cells express Fcε receptor I (FcεRI), a high-affinity immunoglobulin E (IgE) receptor, and stem cell factor (SCF) receptor [c-kit; cluster of differentiation 117 (CD117)] on their cell surface and possess numerous granules containing inflammatory mediators. After IgE-mediated allergen recognition through FcεRI, mast cells not only release these granules but also produce inflammatory mediators such as inflammatory cytokines and chemokines [2]. Finally, these responses result in inflammatory reactions [1, 2].

The role of mast cells in response to radiation responses and the effects of ionizing radiation on mast cells have been studied extensively; in addition, mast cells are relatively radioresistant compared with lymphocytes [3–5]. In contrast, hematopoietic stem/progenitor cells in bone marrow cells (BMCs) are highly radiosensitive [6–11]. In a study using an experimental mouse model of radiation proctitis, mast cell–deficient mice developed lower acute and chronic rectal radiation damage than their control littermates [12], thus indicating the involvement of mast cells in radiation damage. An *in vitro* study using the human mast cell line HMC-1 revealed that ionizing radiation causes degranulation of mast cells [13]. Furthermore, Blirando *et al.* demonstrated the synergistic effects of mast cell–conditioned medium with irradiation in the induction of many inflammatory genes of endothelial cells [14]. These observations suggest that ionizing radiation causes tissue inflammation and injury by presumably modulating mast-cell functions. However, the effects of ionizing radiation on the differentiation of mast cells from their progenitors are unknown.

In this study, to identify the effects of ionizing radiation on the differential induction of mast cells, we investigated whether BMCs from X-irradiated mice could differentiate into mast cells.

MATERIALS AND METHODS

Reagents

L-glutamine, sodium pyruvate, mouse anti-dinitrophenyl IgE (mouse anti-DNP-IgE), dinitrophenyl-human serum albumin (DNP-HSA) and *p*-nitrophenyl-*N*-acetyl-*β*-D-glucosaminide (PNAG) were purchased from Sigma–Aldrich (St Louis, MO, USA). Recombinant human transforming growth factor-β1 (rhTGF-β1) was purchased from Peprotech (Rocky Hill, NJ, USA). Recombinant mouse SCF (rmSCF), recombinant mouse interleukin-3 (rmIL-3) and allophycocyanin (APC)-conjugated anti-mouse FcεRI monoclonal antibody (FcεRI-APC) were purchased from Miltenyi Biotech (Bergisch Gladbach, NRW, Germany). APC-conjugated anti-Armenian hamster immunoglobulin G monoclonal antibody and recombinant mouse interleukin-9 (rmIL-9) were purchased from eBioscience (San Diego, CA, USA). Phycoerythrin (PE)-conjugated anti-mouse CD117 monoclonal antibody (CD117-PE) was purchased from Beckman Coulter (Brea, CA, USA). PE-conjugated anti-rat IgG_{2b} monoclonal antibody was purchased from Becton Dickinson Pharmingen (San Jose, CA, USA). Triton X-100 and glycine were purchased from Wako (Osaka, Japan). Isoflurane was purchased from Mylan Inc. (Pittsburgh, PA, USA).

Animals

C57BL/6JJcl female mouse were purchased from CLEA Japan Inc. (Tokyo, Japan). All bleedings were performed under a 12 h light–dark cycle. The Animal Research Committee of Hirosaki University approved this study (approval number: G12003), which was performed in accordance with the Rules for Animal Experimentation of Hirosaki University.

Irradiation of mice

Eight-week-old mice were exposed to doses of 0.5 Gy or 2 Gy X-radiation. Total-body irradiation (TBI) was performed using an X-ray generator (MBR-1520R-3; Hitachi Medical Co., Tokyo, Japan) under the following conditions: 150 kV, 20 mA, 0.5-mm Al and 0.3-mm Cu filter. Mice were placed 45 cm from the focal point and exposed at a dose rate of 100 cGy/min.

Collection of bone marrow cells

To collect femurs, mice irradiated at eight weeks old were euthanized using isoflurane 1–10 days post-irradiation. BMCs were harvested from both femurs by flushing with 5 mM ethylenediaminetetraacetic acid–phosphate buffered saline (PBS), pH 7.4, containing 0.5% bovine serum albumin. After centrifuging (400*g*, 10 min, 4°C), BMCs were counted using a hemocytometer (Burker–Turk hemocytometer, Sunlead Glass, Saitama, Japan) with Türk's solution (Nacalai Tesque, Kyoto, Japan).

Cell culture

To induce murine bone marrow–derived mast cells (BMMCs), BMCs were cultured in the presence of a cytokine mixture. Briefly, cells (2.0×10^5 cells/ml) were cultured in Dulbecco's modified Eagle medium (DMEM, Sigma–Aldrich) supplemented with 10% heat-inactivated fetal bovine serum (Japan Bioserum Co. Ltd, Hiroshima, Japan), 100 U/ml penicillin, 100 μg/ml streptomycin (Gibco Life Technologies, Carlsbad, CA, USA), 2 mM L-glutamine, 1 mM sodium pyruvate, 5 ng/ml rmIL-3, 5 ng/ml rmIL-9, 1 ng/ml rhTGF-β1 and 50 ng/ml rmSCF at 37°C in a humidified atmosphere containing 5% CO_2. Cells were passaged every week and cultured for 4 weeks.

Cell surface staining

The expression of cell surface antigens was analyzed using a flow cytometer (FACSAria II; Becton Dickinson, Franklin Lakes, NJ, USA). Briefly, cells were harvested, washed with PBS(-) (Wako, Osaka, Japan), and then stained with FcεRI-APC (5 μg/ml) and CD117-PE (5 μg/ml) for 20 min at 4°C in the dark. Cells were also stained with corresponding fluorescence-conjugated isotype-matched control IgGs. The $FcεRI^+$/$c\text{-}kit^+$ cells were regarded as BMMCs.

Measurement of β-hexosaminidase release

To detect granule-localized β-hexosaminidase, an enzymatic colorimetric assay was used, as described previously [15]. Briefly, BMMCs were sensitized overnight with mouse anti-DNP-IgE and then washed with PBS(-) (300*g*, 5 min, 4°C). Following sensitization, BMMCs (3.0×10^4 cells/well) were aliquoted into 96-well flat-bottom plates (Thermo Fisher Scientific Inc., Waltham, MA, USA) and stimulated with DNP-HSA for 30 min at 37°C. Cell-free supernatants were aliquoted into a new plate after centrifuging the plates (300*g*, 5 min, room temperature). The chromogenic substrate PNAG was added to the supernatants and incubated for 90 min at 37°C. A solution of 0.1% Triton X-100 was added into original plate, followed by PNAG. After 90 min incubation at 37°C, the reaction was stopped with 0.4 M glycine and absorbance was measured at 405 nm using a Benchmark Microplate Reader (BioRad, Hercules, CA, USA).

Statistical analysis

Data are represented as the mean ± standard deviation (SD). The comparison between unirradiated and X-irradiated groups was performed using one-way analysis of variance and Dunnett's test. $P < 0.05$ was considered statistically significant. Statistical analysis was performed using Excel 2010 (Microsoft, Redmond, WA, USA) with the add-in software Statcel 3.

RESULTS

The number of bone marrow cells in X-irradiated mice

Because mast cells originate from progenitors that reside in the BMC compartment, we first investigated the effects of X-irradiation on the number of BMCs. As shown in Fig. 1, significant decreases in the number of BMCs were observed 1 day after mice were irradiated at 0.5 Gy or 2 Gy. However, the number of BMCs obtained from irradiated mice gradually recovered, and no significant decrease caused by X-irradiation was observed 5–10 days post irradiation.

Differentiation of BMCs into BMMCs

We next investigated whether BMCs obtained from X-irradiated mice differentiated into BMMCs. We focused on Days 1 and 10 post irradiation because a significant decrease in the number of BMCs after radiation was observed on Day 1, which was completely reversed by

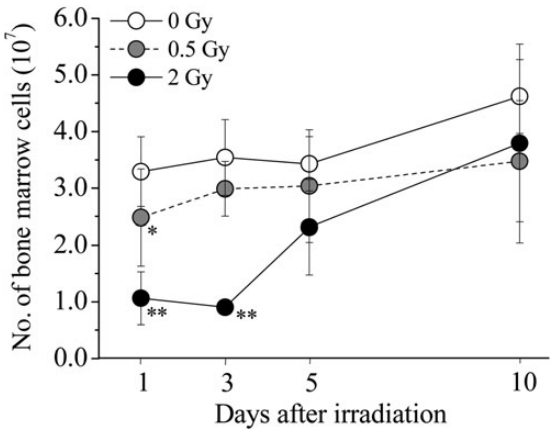

Fig. 1. The number of bone marrow cells in mice exposed to X-irradiation. Mice were exposed to 0.5-Gy or 2-Gy X-irradiation, and bone marrow cells were harvested 1–10 days post-irradiation. The number of bone marrow cells was counted using Türk's solution. Data represent the mean ± SD of at least three different mice. *$P < 0.05$, **$P < 0.0$ (Dunnett's test) compared with unirradiated mice.

Day 10. The cultured BMCs were analyzed using a flow cytometer to confirm the differentiation of BMMCs. Forward scatter (FS) and side scatter (SS) signals indicate cell size and cellular granularity, respectively. As shown in Fig. 2A, FS and SS signals of the induced cells of unirradiated mice markedly increased depending on the culture times, and the cells were large with a high granule content; these are the characteristics of mast cells. Similar results were observed for the cells induced in X-irradiated mice (Fig. 2A). We further analyzed the cell surface expression of FcεRI and c-kit, which are mast cell-related cell-surface antigens (Fig. 2B). The BMCs from both unirradiated and X-irradiated mice moderately expressed c-kit (~60–70%), whereas it hardly expressed FcεRI (~3–4%). After culturing, the percentages of FcεRI$^+$ or c-kit$^+$ cells were increased and FcεRI$^+$/c-kit$^+$ cells (mast cell populations) appeared (Fig. 2B). The percentage of FcεRI$^+$/c-kit$^+$ cells of cultured cells increased with culture time, and this increase was observed in the induced cells from both unirradiated and X-irradiated mice (Fig. 2B). Taken together, these data suggest that the BMCs from X-irradiated mice and unirradiated mice differentiated into mast cells; however, the percentages of c-kit$^+$, FcεRI$^+$ and FcεRI$^+$/c-kit$^+$ cells were significantly lower in X-irradiated mice (Fig. 2C–E).

β-hexosaminidase release from mast cells

To examine the functional characteristics of the mast cells, we determined the release of β-hexosaminidase, which serves as a measure of degranulation, by BMMCs cultured for 4 weeks, because >80% of these cells expressed FcεRI and c-kit. As shown in Fig. 3A, the level of β-hexosaminidase release of BMMCs from X-irradiated mice was significantly higher compared with that of control mice.

To explore the reason for the high β-hexosaminidase release of BMMCs from X-irradiated mice, we investigated the expression levels of FcεRI because it is a high-affinity receptor for IgE. The expression levels of FcεRI were estimated by the mean fluorescence intensity of FcεRI on FcεRI$^+$/c-kit$^+$ cells. As shown in Fig. 3B, the expression levels of FcεRI of BMMCs from X-irradiated mice were lower than or comparable with those from unirradiated mice (Fig. 3B). These results suggest that the high β-hexosaminidase release of BMMCs from X-irradiated mice is not due to the expression levels of FcεRI.

DISCUSSION

Mast cells play a major role in allergic responses and host defense [1]. Although ionizing radiation affects the function of mast cells [12–14], whether ionizing radiation influences the differential induction of mast cells is unknown. In this study, we showed that BMCs from X-irradiated mice differentiated into BMMCs (Fig. 2B). However, the percentage of the cell population expressing mast cell–specific antigens was lower in the X-irradiated mice compared with that in the unirradiated mice (Fig. 2C and D). In contrast, the release of β-hexosaminidase from mast cells shows that the degranulation of BMMCs from X-irradiated BMCs was increased compared with those of unirradiated mice (Fig. 3).

The BMC compartment includes mast cell progenitors as well as myeloid and erythroid progenitors [16, 17]. Meng *et al.* observed that ionizing radiation decreases the number of myeloid progenitors of granulocytes and macrophages [18]. Peslak *et al.* found that the number of burst-forming units was markedly decreased after 1-Gy and 4-Gy TBI [19]. Here we showed that the percentages of mast cells induced in X-irradiated BMCs was lower compared with that in unirradiated BMCs (Fig. 2E), suggesting that ionizing radiation affects mast cell progenitors in a manner similar to that of other hematopoietic cell progenitors.

c-kit is a receptor for SCF playing a crucial role in the differentiation and proliferation of mast cells [2] and is expressed on hematopoietic stem cells and mast cell progenitors [17]. Notably, the percentages of c-kit$^+$ cells of uncultured BMCs or BMCs for 7 days were lower in X-irradiated mice compared with in unirradiated mice (Fig. 2C). Furthermore, in our preliminary experiments, BMCs from mice irradiated at 7 Gy, which causes a lethal hematopoietic syndrome, failed to differentiate into mast cells because the BMCs died during the culture (data not shown). Therefore, it is possible that the low differentiation efficiency of mast cells in X-irradiated mice is likely due to the reduction of mast cell progenitors in BMCs as a result of cell death and senescence after irradiation [20, 21].

Benjamin *et al.* showed that >0.1 Gy irradiation transiently suppresses mast cell degranulation in BMMCs [22]. Furthermore, low-dose radiation (<0.1 Gy) suppresses degranulation of the rat mast cell line Rat Basophilic Leukemia-2H3, which is accompanied by decreases in the expression levels of FcεRI [23]. In contrast, here we showed that degranulation was increased in BMMCs from X-irradiated mice compared with those from unirradiated mice. Because irradiation was applied to mast cells *in vitro*, but not to mast cell progenitors *in vivo*, in previous studies, it is possible that the effects of ionizing radiation on mast cell function differ depending on the cell type (e.g. mature or progenitor cells).

Degranulation of mast cells is triggered by FcεRI-mediated allergen recognition. We showed that the β-hexosaminidase release of BMMCs from X-irradiated mice was higher than that from

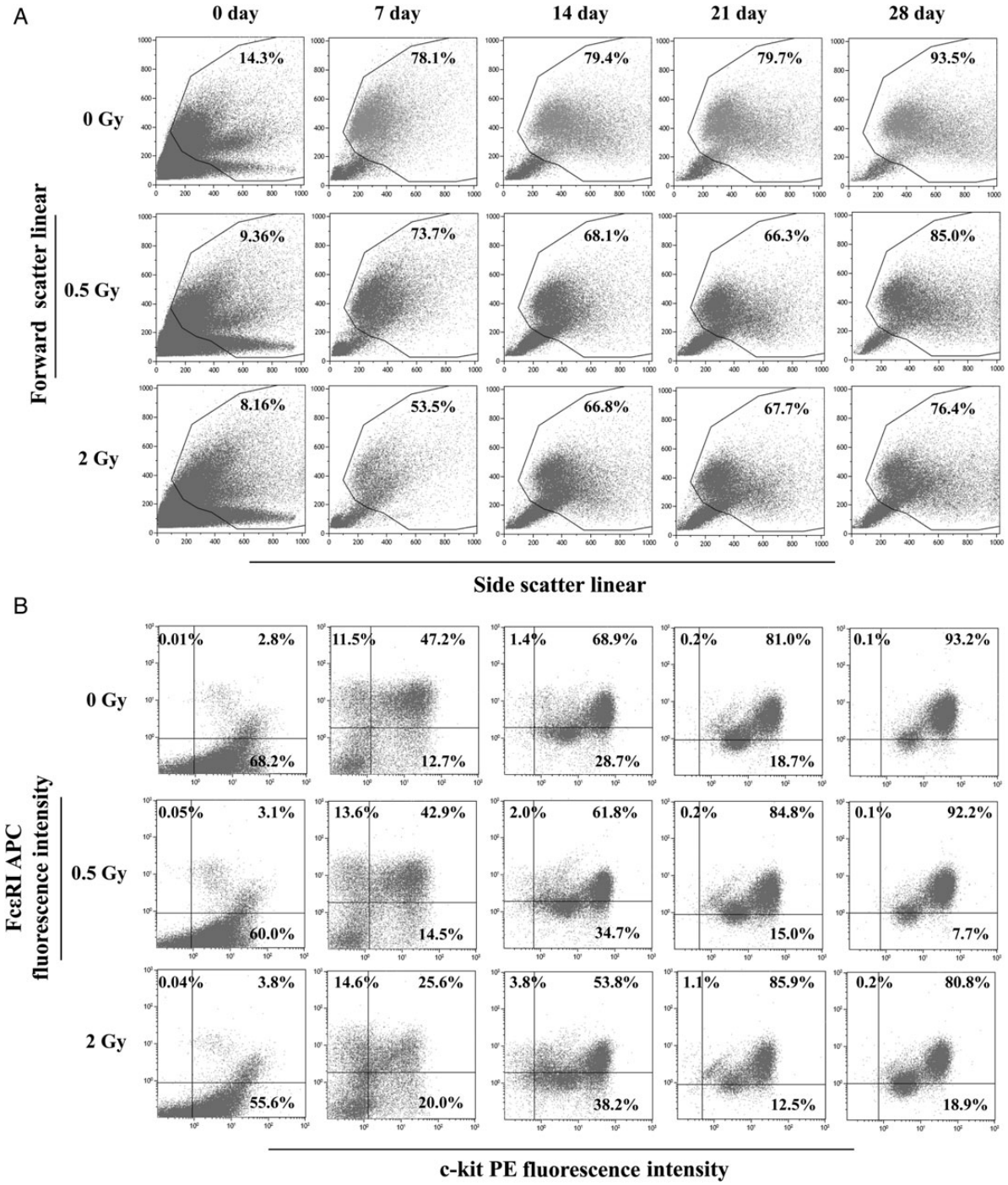

Fig. 2. Expression of FcεRI/c-kit on bone marrow–derived mast cells. (A, B) The bone marrow cells of X-irradiated and unirradiated mice 1 day post-irradiation were cultured for 1–4 weeks. The cultured cells were analyzed using a flow cytometer. (A) Representative forward scatter (FS) and side scatter (SS) panels of cells from non-irradiated mice. (B) After gating as indicated by the FS and SS panels (A), the cell surface expression of FcεRI and c-kit was analyzed. Representative cytograms are shown. The inset numbers represent the percentage of each cell population. (C–E) Bone marrow cells were cultured for 1–4 weeks, and the cultured cells were analyzed using a flow cytometer. The percentages of the c-kit$^+$ (C), FcεRI$^+$ (D), and FcεRI$^+$/c-kit$^+$ (E) populations in cells from unirradiated and X-irradiated mice at 1 (left panel) and 10 (right panel) days post-irradiation are shown. Data represent the mean ± SD of three different mice. *$P < 0.05$, **$P < 0.01$ (Dunnett's test) compared with unirradiated mice.

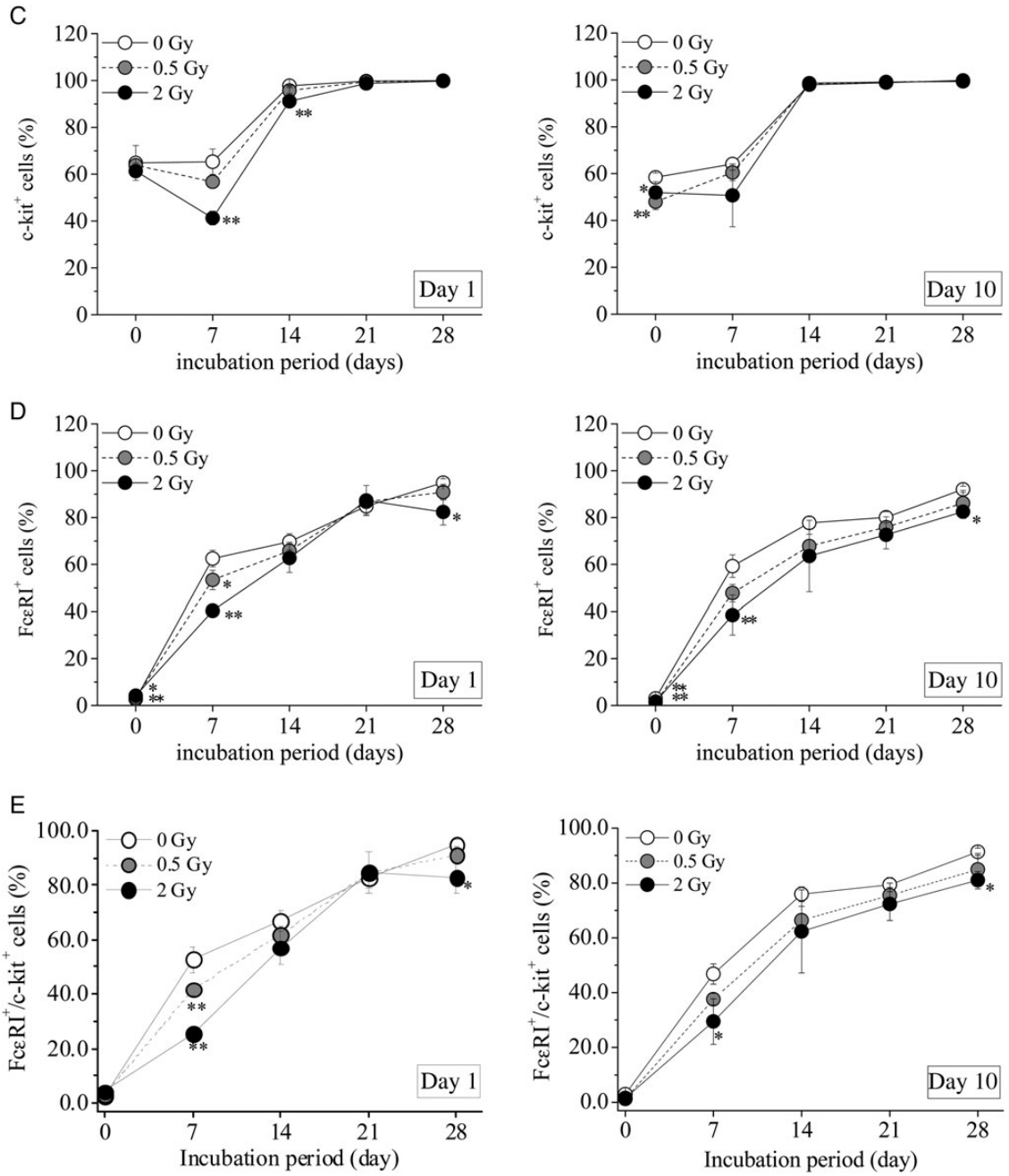

Fig. 2. Continued

unirradiated mice, independent of the expression levels of cell surface FcεRI. Our result is supported by the findings of Ito *et al.* [24]. They showed that the degranulation of murine BMMCs cultured in IL-3 was higher than that of BMMCs cultured in IL-3 plus SCF, although there was no noticeable difference in the expression levels of FcεRI. Their results suggest that the difference of FcεRI-mediated responses between those mast cells is probably due to the expression of Src kinase Hck, which plays a critical role in mast cell activation [25]. Therefore, it is possible that Hck is involved in the high β-hexosaminidase release of BMMCs from X-irradiated mice. Although there have been no previous reports investigating the effects of ionizing radiation on hck expression and activity in detail, hck mRNA is highly expressed in alpha-radiation-induced rat osteosarcomas compared with normal osteoblasts [26]. The involvement of hck in the high β-hexosaminidase release of BMMCs from X-irradiated mice should be examined in future analyses.

Here we demonstrated that X-irradiated mast cell progenitors differentiated into mast cells, although degranulation was enhanced by ionizing radiation. Further studies are required; nevertheless, that the quantitative and qualitative changes in mast cells derived from

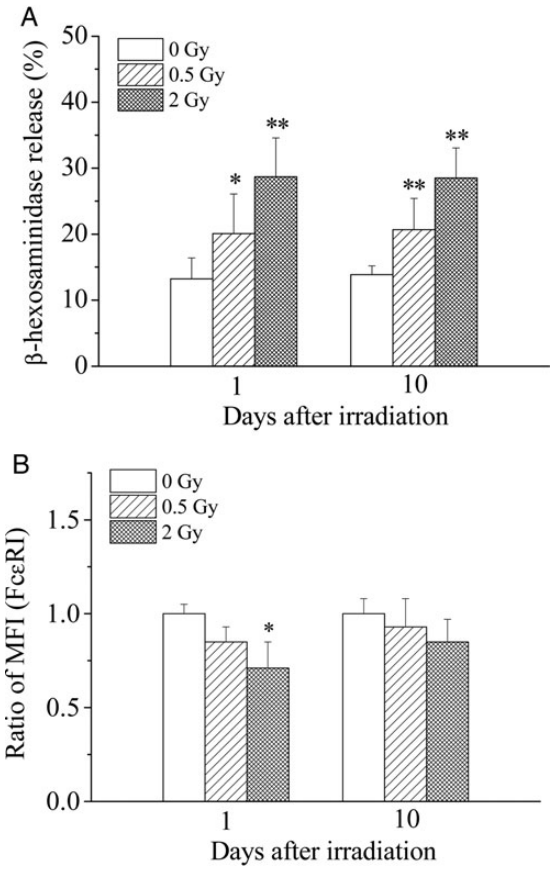

Fig. 3. β-hexosaminidase release after IgE-mediated allergen stimulation. The mast cells were induced from bone marrow cells of X-irradiated and unirradiated mice on days 1 and 10 post-irradiation. (A) The induced cells were stimulated with IgE-mediated allergen, and then the release of β-hexosaminidase was analyzed as described in the Materials and Methods section. Data represent the mean ± SD of triplicate assays of three different mice. *$P < 0.05$, **$P < 0.01$ (Dunnett's test) compared with unirradiated mice. (B) The mean fluorescence intensity (MFI) of FcεRI-APC on FcεRI$^+$/c-kit$^+$ cells was analyzed using a flow cytometer. Results are shown as relative MFI values compared with unirradiated mice. Data represent the mean ± SD of three different mice. *$P < 0.05$ (Dunnett's test) compared with unirradiated mice.

X-irradiated mast cell progenitors may contribute to radiation-induced inflammation and tissue injury.

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REFERENCES

1. Rao KN, Brown MA. Mast cells: multifaceted immune cells with diverse roles in health and disease. *Ann NY Acad Sci* 2008;1143: 83–104.
2. Metcalfe DD, Baram D, Mekori YA. Mast cells. *Physiol Rev* 1997; 77:1033–79.
3. Carter BG. Mast cell numbers and passive cutaneous anaphylaxis in irradiated mice. *Int Arch Allergy Appl Immunol* 1977;54: 378–81.
4. Fukuzumi T, Waki N, Kanakura Y, et al. Differences in irradiation susceptibility and turnover between mucosal and connective tissue-type mast cells of mice. *Exp Hematol* 1990;18:843–7.
5. Price J. Effect of photon (^{60}Co) irradiation on rabbit choroidal mast cells. *Radiat Res* 1975;62:258–66.
6. Imai Y, Nakao I. *In vivo* radiosensitivity and recovery pattern of the hematopoietic precursor cells and stem cells in mouse bone marrow. *Exp Hematol* 1987;15:890–5.
7. Kato K, Omori A, Kashiwakura I. Radiosensitivity of human haematopoietic stem/progenitor cells. *J Radiol Prot* 2013;33:71–80.
8. Kato K, Kuwabara M, Kashiwakura I. The influence of gender-and age-related differences in the radiosensitivity of hematopoietic progenitor cells detected in steady-state human peripheral blood. *J Radiat Res* 2011;52:293–9.
9. Kashiwakura I, Mikinori K, Osamu I, et al. Radiation sensitivity of megakaryocyte colony–forming cells in human placental and umbilical cord blood. *Radiat Res* 2000;153:144–52.
10. Kashiwakura I, Inanami O, Murakami M, et al. Effects of the combination of thrombopoietin with cytokines on the survival of X-irradiated CD34$^+$ megakaryocytic progenitor cells from normal human peripheral bood. *Radiat Res* 2001;158:202–9.
11. Takahashi K, Monzen S, Hayashi N, et al. Correlations of cell surface antigens with individual differences in radiosensitivity in human hematopoietic stem/progenitor cells. *Radiat Res* 2010; 173:184–90.
12. Blirando K, Milliat F, Martelly I, et al. Mast cells are an essential component of human radiation proctitis and contribute to experimental colorectal damage in mice. *Am J Pathol* 2011;178: 640–51.
13. Martin A, Kerstin M, Frank MK, et al. Ionizing radiation induces degranulation of human mast cells and release of tryptase. *Int J Radiat Biol* 2007;83:535–41.
14. Blirando K, Hneino M, Martelly I, et al. Mast cells and ionizing radiation induce a synergistic expression of inflammatory genes in endothelial cells by a mechanism involving p38α MAP kinase and (p65) NF-κB activation. *Radiat Res* 2012;178:556–67.
15. Kuehn HS, Radinger M, Gilfillan AM. Measuring mast cell mediator release. *Curr Protoc Immunol* 2010;91:Unit7.38.1–9.
16. Chen CC, Grimbaldeston MA, Tsai M, et al. Identification of mast cell progenitors in adult mice. *Proc Natl Acad Sci U S A* 2005;102:11408–13.
17. Hallgren J, Gurish MF. Pathways of murine mast cell development and trafficking: tracking the roots and routes of the mast cell. *Immunol Rev* 2007;217:8–18.
18. Meng A, Wang Y, Van ZG, et al. Ionizing radiation and busulfan induce premature senescence in murine bone marrow hematopoietic cells. *Cancer Res* 2003;63:5414–9.

19. Peslak SA, Wenger J, Bemis JC, et al. Sublethal radiation injury uncovers a functional transition during erythroid maturation. *Exp Hematol* 2011;39:434–45.
20. Wang Y, Schulte BA, LaRue AC, et al. Total body irradiation selectively induces murine hematopoietic stem cell senescence. *Blood* 2006;107:358–66.
21. Wang Y, Liu L, Pazhanisamy SK, et al. Total body irradiation causes residual bone marrow injury by induction of persistent oxidative stress in murine hematopoietic stem cells. *Free Radic Biol Med* 2010;48:348–56.
22. Soule BP, Brown JM, Kushnir-Sukhov NM, et al. Effects of gamma radiation on FcεRI and TLR-mediated mast cell activation. *J Immunol* 2007;179:3276–86.
23. Joo HM, Nam SY, Yang KH, et al. The effects of low-dose ionizing radiation in the activated rat basophilic leukemia (RBL-2H3) mast cells. *J Biol Chem* 2012;287:27789–95.
24. Ito T, Smrž D, Jung MY, et al. Stem cell factor programs the mast cell activation phenotype. *J Immunol* 2012;188: 5428–37.
25. Hong H, Kitaura J, Xiao W, et al. The Src family kinase Hck regulates mast cell activation by suppressing an inhibitory Src family kinase Lyn. *Blood* 2007;110:2511–9.
26. Daino K, Ugolin N, Altmeyer-Morel S, et al. Gene expression profiling of alpha-radiation–induced rat osteosarcomas: identification of dysregulated genes involved in radiation-induced tumorigenesis of bone. *Int J Cancer* 2009;125:612–20.