Altered Immune Cell Proportions in the Radiodermatitis Induced Hairless Mice-1 (HR-1)

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Accidental radiation exposures or radiation therapy can cause internal and external damage including radiodermatitis. Even though radiodermatitis is one of the dose limiting factors in radiotherapy, the immunological nature of it is not yet been clearly understood. In this study, we have examined the alteration in immune cell population during the radiodermatitis process. A radiodermatitis model was established in HR-1 mice by locally exposing a posterior dorsal region to 10 Gy X-ray/day for 4 consecutive days. Collagen accumulation, redness, erythema, and dry desquamation of the skin were detected after X-irradiation. The size and total cell number of the spleen decreased immediately after X-irradiation, compared to those of the sham-irradiated mice, and recovered to the normal levels two weeks later. Reduction and recovery of the bone marrow cell population preceded a similar change of the spleen cell population. The proportion of CD4⁺ T cell increased, while the proportion of CD8⁺ T cell decreased ahead of the obvious skin damage, in both lymph node and spleen of the irradiated mice. Interestingly, the proportion of splenic monocytes/macrophages was expanded gradually at a similar kinetics with the aggravation of the radiodermatitis. The infiltration of the CD11b⁺ monocyte/macrophage to the X-irradiated skin also coincided with the development of radiodermatitis. These altered proportions of immune cells may play important roles in radiodermatitis.

INTRODUCTION

Ionizing radiation damages numerous tissues and organs. The skin is an overlying transit tissue and injurious effect on the skin is a common side effect of radiotherapy. Understanding the nature of radiodermatitis is important, as it can be one of the dose limiting factors in radiotherapy.¹–³) Radiodermatitis in human can be classified into acute and chronic types.⁴,⁵) Acute radiodermatitis develops within a few weeks after a high dose of ionizing radiation. The skin shows erythema, epilation, dry- and moist-desquamation, and erosion, with or without hyperpigmentation.⁶) In contrast, chronic radiodermatitis develops years after following repeated low doses of ionizing radiation. Chronic radiodermatitis involves atrophy, telangiectasia, fibrosis, and irregular hyper-/hypo-pigmentation.⁷) It has been shown that AP-1 transcription factor activated by irradiation causes induction of TGF-β (transforming growth factor-β) in the skin, and that the produced TGF-β is a master switch for radiation-induced fibrosis.⁸)

The cells exposed to ionizing irradiation produce reactive oxygen species (ROS).⁹) ROS initiates signal transduction and activates transcription factors such as NF-κB.¹⁰) These change gene expression causing delayed cell cycle, cell apoptosis, and even tumorigenesis.¹¹) Exposure to an ionizing radiation damages hematopoietic system and destroys immune cell precursors.¹²–¹⁵) Harrington et al.¹⁶) reported that splenic mononuclear cell population decreased after whole-body gamma-irradiation. Incompetent immune surveillance and frequent opportunistic infection are frequently observed after irradiation.¹⁸,¹⁶) Immune suppression can be reflected to, or induced by changed proportion of immune cell population in the blood, lymph node, and spleen.¹²,¹⁶,¹⁷)
The association between radiodermatitis and change in immune cell proportions is not clear, yet. This may be partly because there are not many radiodermatitis animal models available. Some investigators have used pig as an experimental animal to study radiodermatitis. However, there are economical and technical difficulties to use pig for experiments. As male Hairless Mice-1 (HR-1) becomes hairless by 4 weeks old, skin change can be easily examined without irritating the skin by shaving. In addition, the skin of HR-1 mice is thin and very close to the human skin. Thus, HR-1 mice have been widely used to study skin tumor, dermatitis, percutaneous absorption, and skin irritation, even though the relationship between hairlessness and immunity is not fully characterized. Here, we established a radiodermatitis model using HR-1 mice by repeated local irradiation of the posterior dorsal region. To verify immune cell types important for radiodermatitis, we analyzed change in immune cell proportions of the peripheral lymphoid organs and checked immune cell infiltration to the skin using the radiodermatitis model.

**MATERIALS AND METHODS**

**Mice**

HR-1 was purchased from Hoshino Laboratory Animal Center (Yashio, Japan). Upon arrival, the HR-1 mice were five weeks old and weighed 20–22g. Four to five mice were housed in each cage and kept under the conventional conditions in a specific pathogen-free (SPF) facility. Mice were adapted to a new environment for 4–6 days before the experiments. Three to nine mice were allocated to each test group and the control group. All of the animal experiments were performed according to the Guidelines for the Care and Use of Laboratory Animals of the Catholic University of Korea. No infection or disease symptom was observed in any of the HR-1 mice during the experiment periods.

**X-irradiation**

Mice were taped down on an acrylic plate and irradiated using a Clinac 2100 C-G accelerator (Varian, Salt Lake City, UT). Posterior dorsal region of HR-1 mouse was exposed to X-irradiation at a dose rate of 4 Gy/min, 10 Gy/day for four consecutive days. Exposure field was fixed by a collimator in the accelerator. Posterior dorsal region was chosen to minimize exposure of vital organs to the irradiation. In addition to the posterior dorsal skin, femur, tibia, and the inguinal lymph nodes were directly exposed to the X-irradiation, while the spleen and the axillary lymph nodes were not exposed. The control mice were sham-irradiated. Skin and immune cell changes of the mice were monitored for 49 days following the irradiation.

**Antibodies**

Fluorescein isothiocyanate (FITC)-conjugated anti-mouse CD4, phycoerythrin (PE)-conjugated anti-mouse CD8, PE-conjugated anti-mouse CD11b, and PE-conjugated anti-mouse CD45/B220 antibodies were used for the experiments. All monoclonal antibodies and isotype controls were obtained from BD Pharmingen (San Diego, CA).

**Tissue preparation and histological staining**

The deposition of dermal collagen, one of the most important dermal matrix components, was examined based on the intensity of trichrome staining. The experimental and control mice were sacrificed at the indicated times after irradiation. Skin samples were fixed in 10% formalin, embedded in wax, and cut into 5–6 μm-thick sections for Masson’s trichrome staining. Tissue sections from all the experimental and control animals were stained simultaneously to avoid variations in the staining conditions between specimens. The trichrome stained sections were analyzed using an optical microscope IX70 (Olympus, Tokyo, Japan) and photographs were taken with a digital camera DP 70 (Olympus).

**Isolation of the cells from lymph node, spleen, and bone marrow**

To collect samples, mice were sacrificed by cervical dislocation at the time described in the results. The axillary and inguinal lymph nodes were removed and minced to make a cell suspension. The lymph node cells were filtered through a cell strainer and centrifuged at 300g for 5 minutes at 4°C. After washed with phosphate buffered saline (PBS), up to 10⁶ cells were resuspended in 100 μl HF2 buffer (Hanks’ balanced salt solution containing 2% fetal bovine serum). The spleens were removed and weighed. Spleen cells were isolated by grinding spleen tissue between a pair of opaque glass slides. Released spleen cells were centrifuged at 300g for 5 minutes at 4°C and resuspended in a RBC lysis buffer (1M NH₄Cl, 0.01M Tris-Cl, pH 7.2–7.4). The cells were then pelleted, washed in PBS, filtered through a cell strainer, and resuspended in the HF2 buffer (< 1 x 10⁶ cells/100 μl). Bone marrow was extracted from two hindlimbs of each mouse. After removing skin, each hindlimb was separated into femur and tibia. Epiphysis was carefully cut away to expose the marrow and a 26-gauge needle was inserted into the distal end. The marrow was washed out with 5 ml RPMI-1640 media and collected in a 50 ml tube. The marrow was broken up into a single-cell suspension by repeated aspiration through a 26-gauge needle. Released bone marrow cells were processed in the same way as the spleen cells.

**FACS analysis of the cells**

The cells were incubated with appropriate fluorescent monoclonal antibodies in the HF2 buffer containing 0.2% propidium iodide (PI) for 30 minutes on ice before washed with PBS. The effect of irradiation on the bone marrow cell viability was analyzed by a FACS Calibur (BD Biosciences, San Jose, CA). The relative proportions of B lymphocytes, T lymphocytes, and viability was analyzed by a FACS Calibur (BD Biosciences, San Jose, CA).

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T lymphocytes, and monocytes/macrophages in the lymph node and spleen were also analyzed with a FACS Calibur (BD Biosciences). Dead cells stained with PI were excluded and only the viable cells were analyzed. Non-specific binding of antibodies was assessed using isotype control antibodies. Five thousand cells were analyzed for each sample, and the results were analyzed using a Cell Quest Software (BD Biosciences).

Detection of CD11b\(^+\) monocytes/macrophages in the skin

The posterior dorsal skins from the sham-irradiated and X-irradiated HR-1 mice were frozen in Tissue-Tec\textsuperscript{®} O.C.T. compound (Sakura Finetek, Torrance, CA). Six-micron sec-

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Fig. 1. Surface macrophotography of the HR-1 mice radiodermatitis model (a), and light micrography of the dorsal skin stained with Masson’s trichrome method (b). The arrow in Fig 1a indicates the region showing severe inflammation.

Fig. 2. The size and cell number of the spleen. Spleen weight (a) and total cell number of the spleen (b) were examined. (c) Spleen size of the sham-irradiated or X-irradiated mice at 6 and 21 days after X-irradiation. C; Sham-irradiated control (○), X; X-irradiated (●). *: P < 0.05
sections were prepared from the frozen tissues. The skin samples were fixed in a 1:1 mixture of acetone and methanol before stained with PE-conjugated anti-mouse CD11b antibody in PBS containing 10% normal donkey serum. Following a 45-min incubation at room temperature, the sections were washed in PBS and observed with a confocal microscope (MRC-1024, Bio-Rad) supplemented with an argon-krypton laser.

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**Fig. 3.** Flow cytometric analysis of bone marrow cells following posterior dorsal region irradiation. The total bone marrow (BM) cell number (a) and the viable cell percents of bone marrow cells (b). C: Sham-irradiated control (○), X: X-irradiated (●). *: P < 0.05

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**Fig. 4.** Relative proportion of T cell subpopulations in the lymph nodes and spleen after X-irradiation. CD4+ T cells (a) and CD8+ T cells (b) in the lymph nodes, CD4+ T cells (c) and CD8+ T cells (d) in the spleen. C: Sham-irradiated control (○), X: X-irradiated (●). *: P < 0.05
Statistics
Cases with a P-value of less than 0.05 were regarded as statistically significant following student t-test.

RESULTS

Induction of radiodermatitis in hairless mice
A single, whole-body X-irradiation at 10 Gy did not induce radiodermatitis, while a single 20 Gy irradiation caused severe weight loss and frequent death of the mice (data not shown). To minimize damage in vital organs, we used local X-irradiation only at a posterior dorsal region of the mice. Dermatitis was induced in HR-1 mice without a significant weight loss by exposing to a repeated 10 Gy/day X-irradiation for 4 consecutive days (Fig. 1a). Erythema in the skin was detectable 10 days after the first X-irradiation. Dry desquamation was observed 12 days after the first X-irradiation. Edema increased progressively until 21 days after X-irradiation, and then decreased compared with the sham-irradiated mice. These acute radiodermatitis symptoms were most severe 18 days after the first irradiation. After this time, the lesion recovered slowly, but was not repaired completely. The skin was still reddish compared with that of the control mouse even 49 days after the first irradiation (Fig. 1a).

The extent of dermal collagen deposition was examined by Masson’s trichrome staining. The dermal collagen fibers appeared more densely packed in the irradiated mice than the sham-irradiated control mice (Fig. 1b). Four days after irradiation, the intensity of trichrome staining in the dermis increased slightly than that of the sham-irradiated mice. Maximum intensity of the staining was observed in the skin at 12 to 21 days after the first irradiation. Staining intensity in the dermis decreased 49 days after the first irradiation, but was still higher than the control level.

Radiation effect on the weight and total cell number of spleen
Following the X-irradiation, spleens and lymph nodes were removed to harvest immune cells at various time points. The weight and total cell number of spleen showed parallel changes (Fig. 2). Significantly reduced spleen

![Graphs showing changes in immune cell populations](https://academic.oup.com/jrr/article-abstract/47/1/9/945678)

Fig. 5. Relative proportion of antigen presenting cell populations in the lymph nodes and spleen after X-irradiation. B220+ B cells (a) and CD11b+ monocytes/macrophages (b) in the lymph nodes, B220+ B cells (c) and CD11b+ monocytes/macrophage (d) in the spleen. C; Sham-irradiated control (○), X; X-irradiated (●). *: P < 0.05
weight and total cell number were observed right after the last irradiation (P < 0.05). The weight and total cell number increased gradually up until 21 days after the first irradiation reaching to the levels of the sham-irradiated mice. The ratio of spleen to body weight showed similar trends with the spleen weight itself (data not shown). Enlarged lymph nodes were noted at around 10–12 days following the first irradiation, and reduced to the normal size later on (data not shown).

Radiation effect on the bone marrow cells

Four days after the first X-irradiation, total cell number of the bone marrow decreased significantly than that of the sham-irradiated mice (X-ray vs. control was 1.9 $\times$ 10^6 vs. 42.4 $\times$ 10^6). The total cell population recovered gradually and reached to the control level 16 days after the first irradiation. Following X-irradiation, the proportion of viable cells in bone marrow decreased continually up until 12th day and began to recover to that of the sham-irradiated mice from 16th day (Fig. 3).

Effect of irradiation on the CD4$^+$ and CD8$^+$ T cell proportion

FACS analysis was performed to characterize the effects of radiation on the T cell subpopulations. Cells isolated from the lymph nodes and spleens were double labeled with FITC anti-mouse CD4 and PE anti-mouse CD8 antibodies. The CD4$^+$ and CD8$^+$ T lymphocyte subsets demonstrated different radiosensitivity in the lymph node and spleen. In lymph nodes, the proportion of CD4$^+$ T cells increased about 15% right after X-irradiation and then slowly decreased compared with that of the sham-irradiated mice. About 21 days after the first X-irradiation, this CD4$^+$ T cell proportion was comparable to that of the sham-irradiated mice (Fig. 4a). In contrast, the proportion of lymph node CD8$^+$ T cell of the irradiated mice decreased around 10% and sustained lower levels for 2 weeks than that of the sham-irradiated mice before recovery (Fig. 4b). In the spleen of irradiated mice, the proportion of CD4$^+$ T cell increased initially about 17% and decreased gradually reaching to the control level after 12 days (Fig. 4c). The proportion of the spleen CD8$^+$ T cells remained near the control level up until 10 days after the first X-irradiation. Ten days after, it decreased about 7% and sustained lower level for a while till it recovered to the level of the sham-irradiated mice by 42nd day (Fig. 4d). Though not statistically significant, proportions of the CD4$^+$ and CD8$^+$ T cells decreased slightly in the blood right after the X-irradiation and then recovered to the control level 1 to 2 weeks later (data not shown).

Effect of irradiation on the B lymphocyte and monocyte/macrophage proportion

The extent of infiltrated CD11b$^+$ monocytes/macrophages to the radiodermatitis induced skin

The extent of infiltrated CD11b$^+$ monocytes/macrophages in the X-irradiated dorsal skin of HR-1 mice was checked by immunohistochemistry. CD11b$^+$ macrophages were easily detected in the dermis of the X-irradiated mice at 21 days after the first irradiation (Fig. 6). The skin collected at 14
days after the first X-irradiation also showed infiltrated CD11b+ cells but at a slightly lower frequency (data not shown). At 4 days after the first irradiation, CD11b+ cells were observed infrequently in the dermis of the X-irradiated mice. Skins of the sham-irradiated mice at 4 days and 21 days showed no infiltration of CD11b+ cells.

DISCUSSION

Accidental radiation exposures or radiation therapy can cause internal and external damage including radiodermatitis.23,24 We have induced radiodermatitis by repeated local X-irradiation and analyzed immune cell changes in the HR-1 hairless mice. The observed acute changes such as erythema and dry desquamation may have been caused primarily from damage to the stem cells of the epidermis and its appendages.6) Activation of p53 by DNA damage following ionizing radiation induces cellular apoptosis or cell cycle arrest at G1/S or G2/M phase.25–26) Immune cells would respond to the damaged epidermis causing the inflammation and erythema as we detected in HR-1 mice.1) The accumulation of collagen appeared as early as 4 days after the first X-irradiation and peaked at 12–21 days. The extent of collagen staining decreased slightly 49 days after the first X-irradiation but still higher than that of the sham-irradiated mice. Whether this early deposition of collagen in the skin is related to the fibrosis process characterized by proliferation of fibroblasts and expansion of collagen fiber found in a chronic radiodermatitis cannot be concluded from our results.

In our experiments, the spleen was not directly exposed to the irradiation, but its size and total cell number shrank rapidly after irradiation. The total cell number and weight of the lymph nodes and spleen can be affected by an inflammation status and a supply of immune cells from the bone marrow.27) In our radiodermatitis animal model, dramatically reduced bone marrow cell population was observed as the hindlimbs as well as inguinal lymph nodes of the HR-1 mice were placed in the radiation fields. The damaged bone marrow must have contributed greatly to the reduced size and cell numbers of the spleen as well as to the decreased blood cell populations (data not shown).

The proportion of CD4+ T cell in both lymph node and spleen increased initially after X-irradiation than that of the sham-irradiated mice, while the proportion of CD8+ T cell showed an opposite profile. These may reflect the relative resistance of the mouse CD4+ T cells to an irradiation compared with CD8+ T cells, as similar increase of the CD4+ T cell proportion was observed in the spleen of whole-body irradiated mice.10) Zan-Bar reported that total lymphoid irradiation causes a long lasting blockage of the B cell maturation process in the spleen.28) The differentiation and maturation of B cells in the lymph node and spleen, supplies plasma cells and memory B cells.29) Following local X-irradiation CD45R/B220+ B cell declined sharply and then recovered later in the spleen and lymph nodes of the HR-1 mice. This might weaken humoral immune reactions and diminish the cytokine production of the B cells. Further study is needed to understand whether these incompetent B cell functions affect radiodermatitis process.

In our radiodermatitis model, the infiltration of the CD11b+ monocyte/macrophage to the X-irradiated skin was most prominent when inflammation of the skin was most severe. CD11b+ monocytes/macrophages are derived from the blood monocyte population,30,31) and are important regulators related to the recruitment and proliferation of the other immune cells. Lorimore et al.32) observed persistent macrophage activation in the spleen and bone marrow of the whole-body irradiated mice. The authors argued that this macrophage activation is a consequence of the recognition and clearance of radiation induced apoptotic cells, rather than a direct effect of irradiation on macrophages. In our experiments, the activated macrophages might have engulfed the damaged cells and proliferated at the X-irradiated skin. These activated macrophages in the damaged skin would recruit other immune cells by secreting cytokines and chemokines. Some of these tissue macrophages may then migrate to the spleen causing increased CD11b+ monocyte/macrophage proportion. These splenic macrophages, in turn, could facilitate B and T cell activation fuelling further immune reaction. It should be noted that the CD11b+ cells we detected in the radiodermatitis-induced HR-1 mice skins may represent not only macrophages but also different populations of immune cells, as CD11b can be expressed on dendritic cells, neutrophils, and some T cells to some extent.33–35)

In summary, we established a radiodermatitis model in hairless mice by repeated local X-irradiation. In the lymph nodes and spleen, each population of immune cells showed differential radiosensitivity. The changes in B220+ B cells and CD4+/CD8+ T cells preceded the induction of radiodermatitis, while the increased splenic CD11b+ monocytes/macrophages appeared to be concomitantly noted with the skin inflammation. In addition, the infiltration of the CD11b+ monocyte/macrophage to the X-irradiated skin peaked when the dermatitis symptoms were most severe. Further studies are needed to clarify the relation of these immune cell derangements to the induction of acute radiodermatitis.

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REFERENCES

1. Bernstein, E. F., Sullivan, F. J., Mitchell, J. B., Salomon, G. D., and Glatstein, E. (1993) Biology of chronic radiation effect on tissues and wound healing. Clin. Plast. Surg. 20: 435–453.

2. Murakami, R., Baba, Y., Nishimura, R., Furusawa, M., Yokoyama, T., Yamashita, Y., Takahashi, M., Yamashita, N., and Ono, T. (1997) The effect of azelastine on acute radiation dermatitis in mice models. Int. J. Radiat. Oncol. Biol. Phys. 37: 907–911.

3. Ertekin, M. V., Tekin, S. B., Erdoğan, F., Karslioğlu, I., Gepdiremen, A., Sezen, O., Balci, E., and Gündoğdu, C. (2004) The effect of zinc sulphate in the prevention of radiation-induced dermatitis. J. Radiat. Res. 45: 543–548.

4. Hopewell, J. W. (1990) The skin: its structure and response to ionizing radiation. Int. J. Radiat. Biol. 57: 751–773.

5. Cipollaro, V. A. (2001) Radiation dermatitis today. J. Eur. Acad. Dermatol. Venereol. 15: 300–301.

6. Malkinson, F. D. and Keane, J. T. (1981) Radiobiology of the radiation dermatitis in mice models. Int. J. Radiat. Oncol. Biol. Phys. 7: 264–265.

7. Kawakami, T., Saito, R., and Miyazaki, S. (1999) Chronic radiodermatitis following repeated percutaneous transiluminal coronary angioplasty. Br. J. Dermatol. 141: 150–153.

8. Martin, M., Lefaix, J. L., and Delanian, S. (2000) TGF-beta 1 and radiation fibrosis: a master switch and a specific therapeutic target? Int. J. Radiat. Oncol. Biol. Phys. 47: 277–290.

9. Pritivirajasingh, S., Story, M. D., Bergh, S. A., Geara, F. B., Ang, K. K., Ismail, S. M., Stevens, C. W., Buchholz, T. A., and Brock, W. A. (2004) Accumulation of the common mitochondrial DNA deletion induced by ionizing radiation. FEBS Lett. 571: 227–232.

10. Wang, T., Zhang, X., and Li, J. J. (2002) The role of NF-kappaB in the regulation of cell stress responses. Int. Immunopharmacol. 2: 1509–1520.

11. Sun, A. Y. and Chen, Y. M. (1998) Oxidative stress and neurodegenerative disorders. J. Biomed. Sci. 5: 401–414.

12. Blomgren, H. and Andersson, B. (1971) Reappearance and relative importance of immunocompetent cells in the thymus, spleen and lymph nodes following lethal X-irradiation and bone marrow reconstitution in mice. J. Immunol. 106: 831–834.

13. Okunewick, J. P., Fulton, D., Markoe, A. M., and Phillips, E. L. (1972) Interrelationship of erythropoietic recovery, marrow recovery, colony-forming units, and erythropoiesis-stimulating factors after sublethal X-irradiation. Radiat. Res. 52: 138–151.

14. El-Naggar, A. M., Hanna, I. R., Chanana, A. D., Carsten, A. L., and Cronkite, E. P. (1980) Bone marrow changes after localized acute and fractionated X irradiation. Radiat. Res. 84: 46–52.

15. Shiraiishi, K., Tachibana, A., Yonezawa, M., and Kodama, S. (2005) Adaptive response of bone marrow stem cells induced by low-dose rate irradiation in C57BL/6 mice. Int. Congr. Ser. 1276: 264–265.

16. Harrington, N. P., Chambers, K. A., Ross, W. M., and Filion, L. G. (1997) Radiation damage and immune suppression in splenic mononuclear cell populations. Clin. Exp. Immunol. 107: 417–424.

17. Chambers, K. A., Harrington, N. P., Ross, W. M., and Filion, L. G. (1998) Relative alterations in blood mononuclear cell populations reflect radiation injury in mice. Cytometry 31: 45–52.

18. Martin, M., Lefaix, J. L., Pintonm, P., Crechet, F., and Daburon, F. (1993) Temporal modulation of TGF-beta 1 and beta-actin gene expression in pig skin and muscular fibrosis after ionizing radiation. Radiat. Res. 134: 63–70.

19. Fujii, M., Tomozawa, J., Mizutani, N., Nabe, T., Danno, K., and Kohno, S. (2005) Atopic dermatitis-like pruritic skin inflammation caused by feeding a special diet to HR-1 hairless mice. Exp. Dermatol. 14: 460–468.

20. Makriou, M., Akamatsu, H., Akita, H., Yamagami, A., Shimizu, Y., Ehiro, H., Kuramoto, M., Suzuki, K., and Matsunaga, K. (2004) Atopic dermatitis-like symptoms in HR-1 hairless mice fed a diet low in magnesium and zinc. J. Int. Med. Res. 32: 392–399.

21. Yamanaka, K., Katsumata, K., Ikuma, K., Hasegawa, A., Nakano, M., and Okada, S. (2000) The role of orally administered dimethylsarsenic acid, a main metabolite of inorganic arsenics, in the promotion and progression of UBV-induced skin tumorigenesis in hairless mice. Cancer Lett. 152: 79–85.

22. Hattori-Nakakuki, Y., Nishigori, C., Okamoto, K., Imamura, S., Hiia, H., and Toyokuni, S. (1994) Formation of 8-hydroxy-2'-deoxyguanosine in epidermis of hairless mice exposed to near-UV. Biochem. Biophys. Res. Commun. 210: 1132–1139.

23. Aslan, G., Terzioglug, A., Tuncali, D., and Bingul, F. (2004) Consequences of radiation accidents. Ann. Plast. Surg. 52: 325–328.

24. Borroni, G., Vassallo, C., Brazzelli, V., Martinoli, S., Ardigo, M., Alessandrino, P. E., Borroni, R. G., and Franchini, P. (2004) Radiation recall dermatitis, panniculitis, and myositis following cyclophosphamide therapy; histopathologic findings of a patient affected by multiple myeloma. Am. J. Dermatopathol. 26: 213–216.

25. Wilgus, T. A., Koki, A. T., Zweifel, B. S., Kusewitt, D. F., Fawwaz, R. A., Reemtsma, K., and Hardy, M. A. (1991) Correlation of TNF-alpha and IL-1 levels in human peripheral blood lymphocytes with in vitro DNA damage. Semin. Cancer. Biol. 435–438.

26. Marcellus-Hoff, M. H. (2005) Integrative radiation carcinogenesis: interactions between cell and tissue responses to DNA damage. Semin. Cancer. Biol. 15: 138–148.

27. Oluwole, S. F., Engelstad, K., De Rosa, C., Wang, T. S., Fawwaz, R. A., Reemtsma, K., and Hardy, M. A. (1991) Migration patterns of dendritic cells and Lymphocytes. Cell Immunol. 133: 390–407.

28. Zan-Bar, I. (1983) Modulation of B and T cell subsets in mice treated with fractionated total lymphoid irradiation. I. Blockage of differentiating B cell pathways. Eur. J. Immunol. 13: 35–40.

29. Byrne, S. N. and Halliday, G. M. (2005) B cells activated in lymph nodes in response to ultraviolet light B-mediated inflammation and tumor formation with topical celecoxib treatment. Mol. Carcinog. 38: 49–58.

30. Gepdiremen, A., Sezen, O., Balci, E., and Gündoğdu, C. (1993) Temporal modulation of TGF-beta 1 and beta-actin gene expression in pig skin and muscular fibrosis after ionizing radiation. Radiat. Res. 134: 63–70.

31. Chambers, K. A., Harrington, N. P., Ross, W. M., and Filion, L. G. (1998) Relative alterations in blood mononuclear cell populations reflect radiation injury in mice. Cytometry 31: 45–52.

32. Martin, M., Lefaix, J. L., Pinton, P., Crecchet, F., and Daburon, F. (1993) Temporal modulation of TGF-beta 1 and beta-actin gene expression in pig skin and muscular fibrosis after ionizing radiation. Radiat. Res. 134: 63–70.

33. Fujii, M., Tomozawa, J., Mizutani, N., Nabe, T., Danno, K., and Kohno, S. (2005) Atopic dermatitis-like pruritic skin inflammation caused by feeding a special diet to HR-1 hairless mice. Exp. Dermatol. 14: 460–468.

34. Makriou, M., Akamatsu, H., Akita, H., Yamagami, A., Shimizu, Y., Ehiro, H., Kuramoto, M., Suzuki, K., and Matsunaga, K. (2004) Atopic dermatitis-like symptoms in HR-1 hairless mice fed a diet low in magnesium and zinc. J. Int. Med. Res. 32: 392–399.
Invest. Dermatol. 124: 570–578.

30. Highton, J., Smith, M., and Bradley, J. (1989) Cells of the monocyte-macrophage series in peripheral blood and synovial fluid in inflammatory arthritis. A preliminary study of cellular phenotype. Scand. J. Rheumatol. 18: 393–398.

31. Valledor, A. F., Borras, F. E., Cullell-Young, M., and Celada, A. (1998) Transcription factors that regulate monocyte/macrophage differentiation. Leukoc. Biol. 63: 405–417.

32. Lorimore, S. A., Coates, P. J., Scobie, G. E., Milne, G., and Wright, E. G. (2001) Inflammatory-type responses after exposure to ionizing radiation in vivo: a mechanism for radiation-induced bystander effects? Oncogene 20: 7085–7095.

33. Koenig, J. M., Matharoo, N., Stegner, J. J., and Schowengerdt, K. O. Jr. (2005) Tacrolimus: In Vitro Effects on Myelopoiesis, Apoptosis, and CD11b Expression. J. Heart Lung Transplant. 24:1332–1336.

34. Maruyama, K., Ii, M., Cursiefen, C., Jackson, D. G., Keino, H., Tomita, M., Van Rooijen, N., Takenaka, H., D’Amore, P. A., Stein-Streilein, J., Losordo, D. W., and Streilein, J.W. (2005) Inflammation-induced lymphangiogenesis in the cornea arises from CD11b-positive macrophages. J. Clin. Invest. 115: 2363–2372.

35. Walton, K. L., He, J., Kelsall, B. L., Sartor, R. B., and Fisher, N. C. (2006) Dendritic cells in germ-free and specific pathogen-free mice have similar phenotypes and in vitro antigen presenting function. Immunol. Lett. 102: 16–24.