Published in final edited form as:

Radiat Res. 2012 September ; 178(3): 166–172.

IL-1 Generated Subsequent to Radiation-Induced Tissue Injury Contributes to the Pathogenesis of Radiodermatitis

Matthew Janko^a, Fernando Ontiveros^a, T. J. Fitzgerald^b, April Deng^a, Maria DeCicco^a, and Kenneth L. Rock^{a,1}

^aDepartment of Pathology, University of Massachusetts, Worcester, Massachusetts 01655

^bDepartment of Radiation Oncology, University of Massachusetts, Worcester, Massachusetts 01655

Abstract

Radiation injury in the skin causes radiodermatitis, a condition in which the skin becomes inflamed and the epidermis can break down. This condition causes significant morbidity and if severe it can be an independent factor that contributes to radiation mortality. Radiodermatitis is seen in some settings of radiotherapy for cancer and is also of concern as a complication post-radiation exposure from accidents or weapons, such as a “dirty bomb”. The pathogenesis of this condition is incompletely understood. Here we have developed a murine model of radiodermatitis wherein the skin is selectively injured by irradiation with high-energy electrons. Using this model we showed that the interleukin-1 (IL-1) pathway plays a significant role in the development of radiodermatitis. Mice that lack either IL-1 or the IL-1 receptor developed less inflammation and less severe pathological changes in their skin, especially at later time-points. These findings suggest that IL-1 pathway may be a potential therapeutic target for reducing the severity of radiodermatitis.

INTRODUCTION

Soon after the discovery of radiation, it became apparent that the skin was a tissue sensitive to radiation damage. Early practitioners of radiography exposed their own hands to X rays for experimentation and calibration, and were the first to notice the consequences of erythema and epilation (1). In fact before instrumentation to measure the absorbed dose of radiation, the development of inflammation of the skin was used to define radiation dosage. One erythema unit was the dose of radiation that was needed to cause skin redness after a single dose of X rays which equates to ~5 Gy (1). High doses and continued exposure led to more serious complications of skin ulceration and gangrene.

At the present time, radiodermatitis continues to be a complication of radiotherapy, e.g., for breast cancer (2). It is also potentially a major complication after exposure to radiation from an accident (3, 4) or terrorist attack, such as a “dirty bomb” (5, 6). For example, in a dirty bomb scenario the alpha and beta particles released may have limited penetration and therefore will primarily damage exposed skin surfaces. After sufficient radiation exposure, damage to the skin can be a major determinant and an independent predictor of mortality (4, 7). Therefore, it is important to understand the underlying mechanisms of radiodermatitis.

Ionizing radiation causes cell injury and death by damaging DNA and other cellular constituents (8). In the skin many cell types can be injured by radiation but damage to the basal cell layer of the epidermis is particularly problematic as it is required for maintaining the structure and integrity of skin. In addition to causing direct damage to cells, radiation to the skin also induces inflammation (9). Some acute inflammation can be seen within hours of exposure, but radiodermatitis begins to develop in earnest 10–14 days after radiation exposure and can progress in severity over the ensuing weeks. In other settings, inflammation alone can cause tissue injury (9, 10). This is because recruited leukocytes release reactive oxygen species, proteases and other toxic molecules that damage adjacent normal cells. Consequently it is thought that in the setting of radiation damage, inflammation is a confounding factor that increases tissue injury and morbidity.

Precisely how radiation of the skin leads to inflammation is not well understood. The injury is sterile and the epidermal barrier to microbial invasion is intact during the time when the inflammatory response develops. Injured and dying cells are known to release proinflammatory molecules called damage-associated molecular patterns (DAMPs) (11, 12) that can stimulate robust inflammation. Since cells are damaged by radiation it is possible that this process underlies the genesis of radiodermatitis. Recent work has shown that sterile inflammation induced by cell death and a number of other particulate stimuli are driven by the cytokine interleukin 1 (IL-1) (12). Therefore, in this report we examined the role of the IL-1 pathway in the pathogenesis of radiodermatitis. We found that when mice that are genetically lacking the IL-1 pathway are exposed to high-energy electrons to the skin they develop less radiodermatitis than wild-type animals post-irradiation.

MATERIALS AND METHODS

Mice

Wild-type male C57BL/6J mice were purchased from Jackson Laboratories (Bar Harbor, ME). IL-1R1-deficient (13), IL-1 α , IL-1 β and IL-1 $\alpha\beta$ double-deficient male mice were described previously (14) and bred at University of Massachusetts Medical School. All mice were maintained on a standard rodent diet at University of Massachusetts Medical School and mice were used at 9–12-weeks of age. Experiments were carried out with University of Massachusetts Medical School Institutional Animal Care and Use Committee (IACUC) approval.

Radiation

Mice were shaved using an electric razor in the flank areas and anesthetized using a 1:10 dilution of pentobarbitone (Nembutal, Hospira, Lake Forest, IL) and DPBS (Gibco, Carlsbad, CA) at a concentration of 30 mg kg⁻¹. Anesthetized mice were placed into a Styrofoam jig with an overlaid 0.8 cm Superflab tissue equivalent material bolus and were irradiated with a 6 MeV beam from a Titanium source using the Varian II Trilogy system (Palo Alto, CA) and a 2 cm circular lead cut-out to direct the beam onto the mouse flank. Total delivered doses ranged from 15–35 Gy with a dose-rate of 10 Gy per minute.

Bioluminescence Imaging

Non-invasive bioluminescence imaging of myeloperoxidase (MPO) activity *in vivo* was used to semi-quantitatively measure inflammation longitudinally, as previously described (15). Mice were anesthetized and given 200 mg kg⁻¹ bioluminescent substrate (Luminol sodium salt, Sigma-Aldrich, St. Louis, MO) diluted in DPBS. Mice were imaged using the IVIS-Xenogen 100 (Caliper Life Sciences) and Living Image software version 4.2 (2 min photon exposure, medium binning, F/Stop 1, open optical filter).

Clinical Evaluation of Skin

Following irradiation mice were grossly inspected several times per week and the quality of the skin was recorded: (0) normal, (1) erythema, (2) dry desquamation, (3) wet desquamation, and (4) ulceration (images shown in Fig. 2). Animals with a score of 4 over a significant area of the skin were euthanized as prescribed by University of Massachusetts Medical School IACUC.

Histology Examination

Mice were euthanized in accordance with IACUC standards and biopsies of skin were obtained. Tissue specimens were frozen, fixed in 10% formalin and embedded in paraffin. Sections were stained with hematoxylin and eosin for histopathological evaluation and rabbit anti-mouse CD31 (Abcam, Cambridge, MA) and ImmPRESS horseradish peroxidase reagent kit (Vector Laboratories, Burlingame, CA) were used for angiogenesis that was analyzed under light microscopy.

Statistical Analysis

Clinical scores and bioluminescence data were analyzed by nonlinear regression analysis (GraphPad, La Jolla, CA) or general linear mixed model analysis (Systat, Chicago, IL; SPSS, ISM, Armonk, NY). Clinical score statistics using a general linear mixed model ANOVA were performed following square root transformation, and bioluminescence statistics using a general linear mixed model ANOVA and curve fitting were performed following natural logarithm transformation to better approximate normal distribution.

RESULTS

Murine Model of Radiodermatitis

Many studies of radiodermatitis in the mouse irradiated the entire hind limb with X rays (16–18) or γ radiation (19–21). Given the depth of penetration of γ rays, the injury in this model is not limited to the skin, but also extends to deeper tissues. We sought to use a model in which the skin would be more selectively exposed to radiation and thereby mimic the injury that would develop after exposure to β particles. To this end the flank skin of C57BL/6J mice was shaved and the right flank was exposed to an electron beam from a Varian II particle accelerator, commonly used in human radiotherapy (22). An advantage of this approach is that the energetic particles have a limited depth of penetration and therefore models damage from β particles. To limit the field of exposure, the beam was directed through a 2 cm lead cutout and to limit the depth of penetration, a 0.8 cm bolus of tissue-equivalent material was placed between the skin and the radiation source. This approach generated a 2 cm high dose area delivered to the skin while significantly limiting the dose to the underlying and surrounding tissue. The contralateral flank was shaved but not exposed to radiation and it served as an internal control.

Treatment doses from 15–35 Gy calculated to the 90% isodose line were used to treat the mice. Mice that were exposed to 20 Gy or greater developed radiodermatitis starting 8–12 days after irradiation and it became severe (clinical scores of 3–4) after ~3 weeks (Fig. 1). Mice that received 15 Gy developed only mild radiodermatitis, while those exposed to 35 Gy developed severe disease more rapidly. Based on these results, we selected single-fraction doses of either 20 or 21.75 Gy for subsequent experiments; the different doses were two variations used by different personnel and similar results were obtained at both doses. The skin was evaluated over time using a scoring system similar to that used by others previously (23) as follows: (0) normal, (1) erythema, (2) dry desquamation, (3) wet desquamation, and (4) ulceration (Fig. 2). Animals with a score of 4 over a significant area of the skin were euthanized as prescribed by our institutional IACUC.

To quantify inflammation in the animals, we used a luminescent imaging assay system. Mice were anesthetized and injected with luminol. This substrate is converted to a luminescent product by myeloperoxidase in neutrophils (15, 24, 25), which can be quantified in living animals with an IVIS imaging system. A major advantage of this system is that the progression of inflammation can be followed over time while concurrently scoring the clinical appearance of the same animals. The inflammation measured by luminol imaging developed with a similar time course and increased in magnitude in parallel to the skin changes observed clinically; inflammation and clinical scores subsequently tended to decrease in those animals that survived >30 days (Figs. 1 and 3). It should be noted that in these graphical displays, the loss of the most severely affected animals from the wild-type group improves this group's average, thereby making the differences between this group and the IL-1R1^{-/-} mice appear smaller, especially at the later time points.

Role of the IL-1 Receptor in Radiodermatitis

To investigate the role of the IL-1 pathway in the development and severity of radiodermatitis, we used a genetic model in which C57BL6 mice lack the IL-1R1. This is the sole receptor for all IL-1 species and therefore mice that lack this receptor are completely unresponsive to this cytokine (26). We found that the IL-1R1-deficient mice began to develop clinical signs of radiodermatitis at the same time as what was observed in wild-type mice and the initial progression of the disease was not different between the two strains (Fig. 3). Beginning approximately 1 week after radiation exposure, local erythema was grossly apparent in all mice. Dry and/or moist desquamation had developed in almost all mice by days 14 and 21, respectively. However, at time points after ~3 weeks, the majority of wild-type mice progressed to grade 4 skin damage and required euthanasia, while the majority of IL-1R1-deficient mice had less severe (3) clinical scores and then tended to recover (Fig. 3). Over this period clinical scores differed significantly between wild-type and IL-1R1^{-/-} mice and this persisted through 42 days, despite the loss of many severely affected wild-type mice at later times (Fig. 3). IL-1R1^{-/-} mice showed less severe inflammation, determined by decreased average clinical scores compared to wild-type mice beginning especially in week 3 after radiation exposure. IL-1R1^{-/-} mice also showed accelerated healing. Quadratic curve estimates of aggregated clinical scores from 3 experiments are summarized in Table 1.

When inflammation was quantified by luminol imaging, IL-1R1^{-/-} mice developed signs of inflammation initially at the same rate as wild-type mice, but did not progress to the same severity of inflammation. IL-1R1^{-/-} mice also had a faster resolution of total inflammation compared to wild-type mice (Fig. 3). There was a significant effect for genotype-side irradiated [$F(4, 364) = 15.39, P < 0.0001$], a significant interaction through all scoring days [$F(4, 382) = 40.04, P < 0.0001$], and a significant interaction through a quadratic estimate of time (day²) that was used to estimate predictably resolving inflammation after 3 weeks [$F(4, 382) = 29.77, P < 0.0001$] (Table 2).

In some experiments lesional and control skin was evaluated histologically and the results were concordant with the clinical scores (Fig. 4). Two weeks after radiation exposure all mice showed similar epidermal atrophy and dermal infiltration of neutrophils, lymphocytes and histiocytes. However, IL-1R1^{-/-} mice were found to have less ulceration, less epidermal atrophy, fewer multinucleated giant cells (MNGC), fewer vessel abnormalities and fewer extravasated red blood cells (RBC) compared to wild-type mice. After 3 weeks, IL-1R1^{-/-} mice were found to have less severe necrosis, more prominent early stages of fibrosis and healing compared to wild-type mice. Additionally some wild-type mice showed markedly elevated inflammatory cells, especially eosinophils, histiocytes, lymphocytes and neutrophils compared to IL-1R1^{-/-} mice. At 4 weeks, most IL-1R1^{-/-} mice showed no ulceration or necrosis of epidermis and showed signs of fibrosis/healing, while almost all

wild-type mice were found to have severe ulceration and moderate necrosis. As in week 3, some wild-type mice showed elevated histiocytes, lymphocytes and neutrophils as compared to IL-1R1^{-/-} mice. After 3 weeks, elevated angiogenesis, increased fibroblasts and marked epidermal and dermal fibrosis were seen in both genotypes by both H&E and CD31+ endothelium immunostain.

Role of IL-1 in Radiodermatitis

The finding that radiodermatitis was less severe in mice lacking IL-1R1 suggested that IL-1 was contributing to the pathogenesis of this condition. To formally test the role of IL-1, we irradiated the skin of mice that were genetically lacking this cytokine (IL-1 α ^{-/-} β ^{-/-} double-deficient mice) and compared their clinical course to wild-type mice. Additionally, to investigate the contribution to radiodermatitis of the different forms of IL-1, we irradiated the skin of mice lacking only IL-1 α or IL-1 β .

There was a significant effect for isotype genotype [F(3, 35.4) = 9.85, $P < 0.0001$], and a significant interaction through all scoring days [F(57, 296) = 8.63, $P < 0.0001$]. Clinical scores began to significantly differ between almost all groups beginning 34 days after irradiation (Table 3). Both IL-1 α - and IL-1 β -deficient mice had milder courses of radiodermatitis relative to wild-type mice. However, only mice lacking IL-1 β differed significantly from wild-type mice. The reduction in their disease was not as dramatic as was observed in mice lacking both forms of IL-1, the clinical score course was similar to that observed in the IL-1R1^{-/-} mice (Fig. 5). These results suggest that both IL-1 isotypes contribute to radiodermatitis, although IL-1 β may play the larger role.

DISCUSSION

We report here that cytokine IL-1 plays an important role in the inflammation that develops in the skin after high-dose radiation injury and contributes to the progressive damage up to and including ulceration. Interestingly, with the radiation dosage we use in these studies the erythema and desquamation that occurs in the skin was not appreciably different by clinical evaluation or bioluminescence monitoring between wild-type mice and IL-1 pathway-deficient mice until 3 weeks after irradiation. Presumably, the earlier changes seen by histology are due to direct radiation damage and/or other inflammatory mediators. However, at later times there was a clear divergence in the inflammation and clinical course between the strains, with the IL-1-deficient mice developing less severe disease.

Our findings build on previous studies where IL-1 was found to be one of the few cytokines that was directly induced after irradiation of the skin (9, 27) and one that continued to be produced at later times at least up to 60 days (9). In addition, IL-1 has been implicated in the development of fibrosis that occurs in chronic radiodermatitis (9). Our results extend these earlier studies by showing a clear role for IL-1 in the pathogenesis of radiodermatitis.

Our findings may have relevance for radiation injury in other tissues. A similar induction of IL-1 has been observed in other tissues after radiation injury. For example, IL-1 α is induced in the murine lung early after radiation and remains elevated for >26 weeks (28). Interestingly, serum IL-1 α levels are elevated and have predictive value in human patients that go on to develop radio-pneumonitis (29). Additionally, IL-1 α and IL-1R-dependent responses to cigarette-smoke-induced neutrophilic inflammation and the development of chronic obstructive pulmonary disease (30). This process may be due to chest exposure to radioactive particles (31), which is considered to be a significant cause of smoking-associated lung cancer. Therefore, IL-1 may have a role in the inflammation and pathology that develops post-radiation injury in many tissues.

The role that IL-1 plays in inflammation post-radiation damage is similar to what has been observed in other settings of cell injury and it is likely these responses are all using similar mechanisms. Injection of a variety of dead cells *in vivo* provokes robust acute inflammation and this response is dependent on IL-1 (32). Similarly, *in situ* damage in organs, e.g., caused by toxic agents, incites inflammation that is dependent on IL-1 (32). In these situations, dying cells expose proinflammatory molecules, referred to as DAMPs, which include uric acid, ATP, HMGB1 and most likely other molecules as well (11, 12). The DAMPs then stimulate macrophages to produce IL-1 (33). We hypothesize that cells injured by radiation release DAMPs over time that then stimulate IL-1-dependent inflammation.

IL-1-dependent sterile inflammation causes tissue injury in many situations. This is seen in responses to sterile irritant particles such as urate crystals in gout (34) and silica particles in silicosis (35). This is also seen after cell death in tissues, where the inflammatory response causes damage to adjacent viable tissue. In all of these situations, it is thought that the tissue damage is caused by reactive and hydrolytic molecules that are released from recruited leukocytes, including reactive oxygen species (36, 37), hypochlorite and proteases (37). Leukocytes are known to be present in radiodermatitis and our imaging findings and histological analyses show that the accumulation of neutrophils in these lesions is somewhat dependent on IL-1. Therefore, it is possible that IL-1-dependent leukocyte-mediated damage similarly contributes to the pathogenesis of radiodermatitis.

The present findings are of potential relevance because they give insights into some of the mechanisms of radiodermatitis. Perhaps more importantly, they point to the IL-1 pathway as potential molecular targets for therapy. This is of particular interest because there are already a number of biologics (IL-1Ra, anti-IL-1 and IL-1-trap) that block this cytokine and that are approved for use in humans in other indications (38, 39).

The animal model we developed for these studies should also be useful for future investigations. The electron beam used to irradiate the skin has very limited penetration into deeper tissues, and this models the situation that would occur with surface exposure to β or α particles better than with previous models of γ or X irradiation. Moreover, since damage is limited to the skin, this model allows an analysis of radiodermatitis that limits confounding changes from damage to deeper structures. In addition, the use of luminol-based imaging allows inflammation to be monitored in living animals. Since animals don't have to be sacrificed with this non-invasive technique, the course of disease can be followed in individual mice over an extended time course.

Acknowledgments

This work was supported by grants 1RC1AI081281-01 from the National Institute of Allergy and Infectious Diseases to KLR. Core resources from the Diabetes Endocrinology Research Center grant DK32520 to University of Massachusetts Medical School were used. We would like to thank Yu Liu and the DERC Lab at the University of Massachusetts Medical School for slide preparation, Stephen Baker for statistical analysis assistance, Jonathan Saleeby, Diane Safer, and Linda Ding at the University of Massachusetts Memorial Radiation Oncology Department, Hajime Kono for technical assistance, and Sharlene Hubbard and University of Massachusetts Medical School Animal Medicine for mice maintenance.

References

1. Fitzgerald TJ, Jodoin MB, Tillman G, Aronowitz J, Pieters R, et al. Radiation therapy toxicity to the skin. *Dermatol Clin*. 2008; 26(1):161–72. [PubMed: 18023776]
2. Chen MF, Chen WC, Lai CH, Hung CH, Liu KC, Cheng YH. Predictive factors of radiation-induced skin toxicity in breast cancer patients. *BMC Cancer*. 2010; 10:508. [PubMed: 20860847]

3. Akiba S. Epidemiological studies of Fukushima residents exposed to ionising radiation from the Fukushima Daiichi Nuclear Power Plant prefecture-a preliminary review of current plans. *J Radiol Prot.* 2012; 32(1):1–10. [PubMed: 22327057]
4. Steinert M, Weiss M, Gottlöber P, Belyi D, Gergel O, et al. Delayed effects of accidental cutaneous radiation exposure: fifteen years of follow-up after the Chernobyl accident. *J Am Acad Dermatol.* 2003; 49(3):417–23. [PubMed: 12963904]
5. Williams JP, Brown SL, Georges GE, Hauer-Jensen M, Hill RP, et al. Animal models for medical countermeasures to radiation exposure. *Radiat Res.* 2010; 173(4):557–78. [PubMed: 20334528]
6. Valentin J. Protecting people against radiation exposure in the event of a radiological attack. A report of The International Commission on Radiological Protection. *Ann ICRP.* 2005; 35(1):1–110.
7. Hymes SR, Strom EA, Fife C. Radiation dermatitis: clinical presentation, pathophysiology, and treatment 2006. *J Am Acad Dermatol.* 2006; 54(1):28–46. [PubMed: 16384753]
8. Fajardo, LF.; Berthrong, M.; Anderson, RE. *Radiation Pathology.* New York: Oxford University Press; 2001.
9. Liu W, Ding I, Chen K, Olschowka J, Xu J, et al. Interleukin 1beta (IL1B) signaling is a critical component of radiation-induced skin fibrosis. *Radiat Res.* 2006; 165(2):181–91. [PubMed: 16435917]
10. Rock KL, Latz E, Ontiveros F, Kono H. The sterile inflammatory response. *Annu Rev Immunol.* 2010; 28:321–42. [PubMed: 20307211]
11. McDonald B, Pittman K, Menezes GB, Hirota SA, Slaba I, et al. Intravascular danger signals guide neutrophils to sites of sterile inflammation. *Science.* 2010; 330(6002):362–6. [PubMed: 20947763]
12. Rock KL, Lai JJ, Kono H. Innate and adaptive immune responses to cell death. *Immunol Rev.* 2011; 243(1):191–205. [PubMed: 21884177]
13. Glaccum MB, Stocking KL, Charrier K, Smith JL, Willis CR, et al. Phenotypic and functional characterization of mice that lack the type I receptor for IL-1. *J Immunol.* 1997; 159(7):3364–71. [PubMed: 9317135]
14. Horai R, Asano M, Sudo K, Kanuka H, Suzuki M, et al. Production of mice deficient in genes for interleukin (IL)-1alpha, IL-1beta, IL-1alpha/beta, and IL-1 receptor antagonist shows that IL-1beta is crucial in turpentine-induced fever development and glucocorticoid secretion. *J Exp Med.* 1998; 187(9):1463–75. [PubMed: 9565638]
15. Gross S, Gammon ST, Moss BL, Rauch D, Harding J, et al. Bioluminescence imaging of myeloperoxidase activity in vivo. *Nat Med.* 2009; 15(4):455–61. [PubMed: 19305414]
16. Zhong N, Morris GM, Bacarian T, Rosen EM, Dilmanian FA. Response of rat skin to high-dose unidirectional X-ray micro-beams: a histological study. *Radiat Res.* 2003; 160(2):133–42. [PubMed: 12859223]
17. Lee YS, Mollah ML, Sohn KC, Shi G, Kim DH, et al. ID3 mediates X-ray-induced apoptosis of keratinocytes through the regulation of beta-catenin. *J Dermatol Sci.* 2010; 60(3):138–42. [PubMed: 21030215]
18. Lowe AS, Walker MD, O'Byrne M, Baxter GD, Hirst DG. Effect of low intensity monochromatic light therapy (890 nm) on a radiation-impaired, wound-healing model in murine skin. *Lasers Surg Med.* 1998; 23(5):291–8. [PubMed: 9888325]
19. Moriyasu S, Yamamoto K, Kureyama N, Okamura K, Ikeda T, Yamatodani A. Involvement of histamine released from mast cells in acute radiation dermatitis in mice. *J Pharmacol Sci.* 2007; 104(2):187–90. [PubMed: 17558180]
20. Flanders KC, Sullivan CD, Fujii M, Sowers A, Anzano MA, et al. Mice lacking Smad3 are protected against cutaneous injury induced by ionizing radiation. *Am J Pathol.* 2002; 160(3):1057–68. [PubMed: 11891202]
21. Flanders KC, Major CD, Arabshahi A, Aburime EE, Okada MH, et al. Interference with transforming growth factor-beta/Smad3 signaling results in accelerated healing of wounds in previously irradiated skin. *Am J Pathol.* 2003; 163(6):2247–57. [PubMed: 14633599]
22. Liu W, Qian J, Hancock SL, Xing L, Luxton G. Clinical development of a failure detection-based online repositioning strategy for prostate IMRT-experiments, simulation, and dosimetry study. *Med Phys.* 2010; 37(10):5287–97. [PubMed: 21089763]

23. Ohta T, Iwakawa M, Oohira C, Noda S, Minfu Y, et al. Fractionated irradiation augments inter-strain variation of skin reactions among three strains of mice. *J Radiat Res.* 2004; 45(4):515–9. [PubMed: 15635260]
24. Rinaldi M, Moroni P, Paape MJ, Bannerman DD. Differential alterations in the ability of bovine neutrophils to generate extracellular and intracellular reactive oxygen species during the periparturient period. *Vet J.* 2008; 178(2):208–13. [PubMed: 17869554]
25. Lundqvist H, Dahlgren C. Isoluminol-enhanced chemiluminescence: a sensitive method to study the release of superoxide anion from human neutrophils. *Free Radic Biol Med.* 1996; 20(6):785–92. [PubMed: 8728025]
26. Dinarello CA. Biologic basis for interleukin-1 in disease. *Blood.* 1996; 87(6):2095–147. [PubMed: 8630372]
27. Xiao Z, Su Y, Yang S, Yin L, Wang W, et al. Protective effect of esculentoside A on radiation-induced dermatitis and fibrosis. *Int J Radiat Oncol Biol Phys.* 2006; 65(3):882–9. [PubMed: 16751070]
28. Rubin P, Johnston CJ, Williams JP, McDonald S, Finkelstein JN. A perpetual cascade of cytokines postirradiation leads to pulmonary fibrosis. *Int J Radiat Oncol Biol Phys.* 1995; 33(1):99–109. [PubMed: 7642437]
29. Chen Y, Hyrien O, Williams J, Okunieff P, Smudzin T, Rubin P. Interleukin (IL)-1A and IL-6: applications to the predictive diagnostic testing of radiation pneumonitis. *Int J Radiat Oncol Biol Phys.* 2005; 62(1):260–6. [PubMed: 15850931]
30. Botelho FM, Bauer CM, Finch D, Nikota JK, Zavitz CC, et al. IL-1alpha/IL-1R1 expression in chronic obstructive pulmonary disease and mechanistic relevance to smoke-induced neutrophilia in mice. *PLoS One.* 2011; 6(12):e28457. [PubMed: 22163019]
31. Shousha HA, Ahmad F. Natural Radioactivity contents in tobacco and radiation dose induced from smoking. *Radiat Prot Dosimetry.* 2012; 150(1):91–5. [PubMed: 21926418]
32. Chen CJ, Kono H, Golenbock D, Reed G, Akira S, Rock KL. Identification of a key pathway required for the sterile inflammatory response triggered by dying cells. *Nat Med.* 2007; 13(7):851–6. [PubMed: 17572686]
33. Kono H, Karmarkar D, Iwakura Y, Rock KL. Identification of the cellular sensor that stimulates the inflammatory response to sterile cell death. *J Immunol.* 2010; 184(8):4470–8. [PubMed: 20220089]
34. Kono H, Chen CJ, Ontiveros F, Rock KL. Uric acid promotes an acute inflammatory response to sterile cell death in mice. *J Clin Invest.* 2010; 120(6):1939–49. [PubMed: 20501947]
35. Hornung V, Bauernfeind F, Halle A, Samstad EO, Kono H, et al. Silica crystals and aluminum salts activate the NALP3 inflammasome through phagosomal destabilization. *Nat Immunol.* 2008; 9(8):847–56. [PubMed: 18604214]
36. Komatsu J, Koyama H, Maeda N, Aratani Y. Earlier onset of neutrophil-mediated inflammation in the ultraviolet-exposed skin of mice deficient in myeloperoxidase and NADPH oxidase. *Inflamm Res.* 2006; 55(5):200–6. [PubMed: 16830107]
37. Majno G, Joris I. *Cells, tissues, and disease principles of general pathology.* New York: Oxford University Press; 2004.
38. Braddock M, Quinn A. Targeting IL-1 in inflammatory disease: new opportunities for therapeutic intervention. *Nat Rev Drug Discov.* 2004; 3(4):330–9. [PubMed: 15060528]
39. Dinarello CA. Blocking IL-1 in systemic inflammation. *J Exp Med.* 2005; 201(9):1355–9. [PubMed: 15867089]

Surviving Animals

▲ 1500cGy n=4

● 2000cGy n=6

× 2500cGy n=2

◆ 3000cGy n=2

▼ 3500cGy n=2

3 3
3 3
0 0
0

FIG. 1.

Clinical evaluation of radiodermatitis as a function of radiation dose. The skin of the right flank of C57BL6 mice was exposed to the indicated doses of radiation and then evaluated clinically over time. Fifty percent of C57BL6 wild-type mice receiving 2,000 cGy to right flank skin developed grade 4 skin damage 30 days after exposure. Animals were sacrificed if they developed a skin score of 4 (ulceration). The number of surviving animals over time is shown at the top of the graph. Data are shown as mean values $\pm$ SEM.

FIG. 2. Gross photographs demonstrate different skin changes after ionizing radiation exposure in C57BL6 mice. Panel A: Normal-appearing skin, score 0; panel B: erythema, score 1; panel C: dry desquamation, score 2; panel D: wet desquamation, score 3; and panel E: ulceration, score 4.

FIG. 3.

Course of radiodermatitis in wild-type versus IL-1R1-deficient mice. Skin of C57BL6 and IL-1R1-deficient mice was exposed to 2,175 cGy ionizing radiation and changes were recorded over time by clinical observation (panel A) and bioluminescence monitoring (panel B). Animals were sacrificed if they developed a skin score of 4 (ulceration). The number of surviving animals over time is shown at the top of the graph. Data are shown as mean values $\pm$ SEM (representative experiment).

FIG. 4.

(Immuno)histological images irradiated skin. Photomicrographs of skin sections from representative wild-type (panels A, C, E) or IL-1R1^{-/-} (panels B, D, F) mice exposed to 2,000 cGy. Panels A–D were stained with H&E or with anti-CD31 (panels E, F) either 2 weeks (panels A, B) or 3 weeks (panels C, D) post-irradiation. Magnification is 20× for the main images, and 10× for the insets in panels A and B, and 4× for the insets in panels C and D.

Surviving Animals

● <i>Wt B6</i> <i>n</i> =15	13	5	5
△ <i>IL-1α</i> ^{-/-} <i>n</i> =10	9	8	7
▽ <i>IL-1β</i> ^{-/-} <i>n</i> =16		14	13
◇ <i>IL-1α</i> ^{-/-} <i>β</i> ^{-/-} <i>n</i> =16	14		14

FIG. 5.

Course of radiodermatitis in wild-type versus IL-1 deficient mice. Skin of C57BL6 and IL-1-deficient mice was exposed to 2,000 cGy ionizing radiation and changes were recorded over time by clinical observation. Animals were sacrificed if they developed a skin score of 4 (ulceration). The number of surviving animals over time is shown at the top of the graph. Data are shown as mean values $\pm$ SEM.

TABLE 1Summary of Clinical Evaluation Differences Between Wild-Type and IL-1R1^{-/-} Mice Through 42 Days

Quadratic regression analysis best-fit values	Wild-type (<i>n</i> = 27)	IL-1R1 ^{-/-} (<i>n</i> = 32)
B0, 95% CI	-0.7717 ± 0.1816	-0.6784 ± 0.1880
B1, 95% CI	0.2077 ± 0.01900	0.2054 ± 0.01980
B2, 95% CI	-0.002400 ± 0.0003450 *	-0.003300 ± 0.006253 *

* Significance.

TABLE 2

Summary of Longitudinal Bioluminescence Monitoring Statistical Curve Fitting for Wild-Type and IL-1R1^{-/-} Mice after Ionizing Radiation Exposure

Effect interaction	Estimate for fixed effect	Pr > t , df
Day*wild-type*irradiated side	0.458	<0.001 ***, 390
Day*IL-1R1 ^{-/-} *irradiated side	0.246	<0.001 ***, 384
Day*wild-type*nonirradiated side	0.009	0.842 (n.s.), 390
Day*IL-1R1 ^{-/-} *nonirradiated side	0.0128	0.691 (n.s.), 384
Day ² *wild-type*irradiated side	-0.00911	<0.001 ***, 391
Day ² *IL-1R1 ^{-/-} *irradiated side	-0.00516	<0.001 ***, 383
Day ² *wild-type*nonirradiated side	-0.000210	0.855 (n.s.), 390
Day ² *IL-1R1 ^{-/-} *nonirradiated side	-0.000750	0.291 (n.s.), 383

TABLE 3

Summary of Clinical Evaluation Differences Between IL-1-deficient and Wild-Type Mice

Genotypes	Day	Differences of least squares means, <i>P</i> value (Tukey-Kramer adjusted), <i>df</i> , sig*
Wild-type, IL-1 $\alpha^{-/-}$ $\beta^{-/-}$	34	<i>P</i> <0.001, 194, ***
Wild-type, IL-1 $\alpha^{-/-}$	40	<i>P</i> = 0.9804, 260, n.s.
Wild-type, IL-1 $\beta^{-/-}$	34	<i>P</i> <0.001, 217, ***
IL-1 $\alpha^{-/-}$, IL-1 $\beta^{-/-}$	34	<i>P</i> <0.001, 183, ***
IL-1 $\alpha^{-/-}$, IL-1 $\alpha^{-/-}$ $\beta^{-/-}$	34	<i>P</i> = 0.04, 182, *
IL-1 $\beta^{-/-}$, IL-1 $\alpha^{-/-}$ $\beta^{-/-}$	34	<i>P</i> <0.001, 218, ***