Effects of Low- or Moderate-dose Whole Body-X-ray Radiation on the Immune System of C57BL/6 Mice

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Background: Increase in the use of diagnostic imaging or occupational exposure to radiation have brought upon concerns on the safety and biological effects of low- or moderate-dose radiation. However, limited information is available on the effects of low or moderate dose radiation on human health.

Methods: Using C57BL/6 mice, we aimed to evaluate the biological effects of low- and moderate-dose radiation on the immune system. X-rays was chosen as a radiation source and we analyzed complete blood counts, various lymphocyte subsets and various cytokine levels after single fraction x-ray exposure (0.1 Gy, 1 Gy).

Results: No significant changes in the immunologic parameter of C57BL/6 mice were observed after radiation, except LIX (a cytokine equivalent to human CXCL5), that showed higher level after 0.1 Gy radiation compared to the control.

Conclusion: We observed that a single fraction of low or moderate dose of X-ray radiation does not cause significant changes in the immune system of C57BL/6 mice. Further studies are necessary to elucidate the mechanism underlying our results.

Key Words: Low-dose, X-ray, Radiation, Immune system, Mice
Low/Moderate Dose X-ray Radiation and the Immune System of C57BL/6 Mice

Epidemiological studies of residents in high-background radiation areas have demonstrated that levels of natural killer (NK) cells were higher in the peripheral blood of this population [14]. Moreover, the incidence of cancer in this population was not different from that in other areas. The immune system responds to exogenous pathogens or environmental hazards. It has been suggested that exposure to ionizing radiation modulates immune responses in a complex dose-dependent pattern, while high-dose radiation severely affects hematopoietic cells and suppresses the immune system [15-17]. Furthermore, a low or moderate dose of radiation has been applied for the treatment of benign inflammatory and hyper-proliferative diseases in the past decades [18,19].

In this study, we analyzed the biological effects of low- and moderate-dose radiation on the immune system of C57BL/6 mice. We chose X-rays as a radiation source, since medical exposures are more prevalent in the general population, and selected two radiation doses: 0.1 and 1 Gy (low and moderate doses, respectively). The effects of X-ray radiation were analyzed using complete blood counts and various lymphocyte subsets including NK cells, and changes in various cytokine levels after low and moderate X-ray radiation were analyzed.

**Materials and Methods**

1) Mice and irradiation

C57BL/6 mice were purchased from Orient Bio, Inc. (Seoul, Republic of Korea) and were bred as a homozygous line. They were maintained under specific pathogen-free conditions at the Laboratory of Animal Research Center in Korea Institute of Radiological Medical Sciences (Seoul, Republic of Korea). Experiments were conducted according to the guidelines for ethical use of animals of our institution under an approved protocol. After acclimatization, 9 to 10-week-old mice were individually placed in plastic cages and were exposed to radiation using X-RAD 320 Biological Irradiator (Precision Xray, Inc., CT, USA) at a dose rate 0.1 Gy/min. Radiation doses were 0.1 (N=6) and 1 Gy (N=6). Two mice were used as sham control. The mice were sacrificed for analysis 96 h after exposure to the radiation. Experiments were conducted according to the guidelines for ethical use of animals of our Institution under an approved protocol (KIRAMS 2015-0018).

**Fig. 1.** Comparison of blood cells counts between 0.1 and 1 Gy radiation. The data are means±SEM (*P<0.05).
Fig. 2. Changes of immune cells after low and moderate dose radiation. The data are presented as means±SEM (*P<0.05).
2) Analysis of blood, bone marrow, and spleen after radiation exposure

All blood samples were collected from the inferior vena cava under anesthesia and immediately placed in an EDTA-coated tube. Blood counts were analyzed using the VetScan HM5 analyzer (Abaxis, CA, USA), and functional cytokine expressions were measured by using a Mouse Inflammation Array kit (RayBiotech, GA, USA) according to the manufacturer’s protocol. Mononuclear cells were isolated from the bone marrow and spleen, and single-cell suspensions were prepared by standard procedures. Cells were stained with the following antibodies: mCD3-fluorescein isothiocyanate (FITC), mCD4-phycocyanin (PE), mCD8-allophycocyanin (APC), mCD19-allophycocyanin (APC), mCD11c-allophycocyanin (APC), mCD25-fluorescein isothiocyanate (FITC), mNK-1.1-phycocyanin (PE), and mI-A/I-E (MHC II)-phycocyanin (PE). All of the antibodies were purchased from BD Biosciences (CA, USA). Flow cytometry was performed on FACSCanto II (BD Biosciences, CA, USA) and $10^5$ to $10^7$ events were acquired per sample and analyzed using FlowJo software (FlowJo LLC, OR, USA).

3) Statistical analysis

Statistical analysis was performed using GraphPad Prism software (GraphPad Software Inc., CA, USA) and SPSS (IBM Corp., NY, USA), and $P<0.05$ was considered statistically significant.

Fig. 3. Radiation-induced cytokine changes in the peripheral blood of C57BL/6 mice. The data are presented as means±SEM ($^aP<0.05$).
Results

After a single exposure to radiation, various hematological indices were analyzed and compared with the sham control. We could not observe any significant differences in hematologic indices after 0.1 Gy radiation. The mice that received 1 Gy of radiation had a higher neutrophil count and lower lymphocyte count than the control mice, however, these hematological values stay within the normal reference range (Fig. 1). T subsets, B-cells, natural killer (NK) cells, and dendritic cells (DC) from the bone marrow and spleen were analyzed. In mice that received 0.1 Gy of radiation, the proportion of CD3^+ T cells were higher in the spleen than in the bone marrow (Fig. 2). Overall, we could not observe any significant differences in the proportion of CD4^+ helper T, CD8^+ cytotoxic T, CD4^+ CD25^+ Treg, CD11c^+MHCI^+ dendritic, and NK 1.1^+ NK cells in mice that received 0.1 or 1 Gy of radiation (Fig. 2). Furthermore, the proportion of CD19^+ B cells was not significantly different in the irradiated mice when compared to that in control mice. Levels of interferon (IFN)-γ, tumor necrosis factor (TNF)-R1, interleukin (IL)-1β, IL-2, IL-4, IL-6, IL-10 and lipopolysaccharide-induced CXC chemokine (LIX) were analyzed after exposure to radiation (Fig. 3). Except for LIX, the expression levels of these cytokines were not significantly different between the control mice and mice that received either 0.1 or 1 Gy of X-ray radiation. Interestingly, mice that received 0.1 Gy of radiation had higher levels of LIX (mouse equivalent of CXCL5) than control mice. However, the levels of LIX in mice irradiated with 1 Gy were not significantly different from those in control mice.

Discussion

In this study, we analyzed the effect of a single fraction of 0.1 and 1 Gy dose of X-ray radiation on the immune system of 9-10-week-old female C57BL/6 mice. Overall, no significant differences were observed in hematologic indices in the peripheral blood of control and irradiated mice. A low dose of radiation appeared to increase the fraction of CD3^+ T cells, as mice irradiated with 0.1 Gy of radiation had a higher proportion of CD3^+ T cells in the spleen. Unexpectedly, the expression levels of LIX, an inflammatory cytokine, was higher in mice that received 0.1 Gy, while no differences were observed in mice irradiated with 1 Gy of radiation.

It has been suggested that low-dose radiation has anti-inflammatory effect, while moderate doses have pro-inflammatory effects. According to the definition of United Nations Science Committee on the Effects of Atomic Radiation (UNSCEAR), low-dose radiation refers to doses of equal to or less than 0.1 Gy. Unexpectedly, we observed that mice that received a single exposure to 0.1 Gy X-ray had higher levels pro-inflammatory cytokine LIX compared to control mice. However, mice that received 1 Gy of radiation did not show any differences in the expression levels of LIX. LIX is an inflammatory cytokine equivalent to human CXCL5, and is involved in neutrophil induction as well as recruitment and activation of white blood cells. A recent study identified CXCL5 as a peripheral mediator of UVB-induced inflammatory pain in rat and human skin [20,21]. Human CXCL5 is secreted by inflammatory cells such as neutrophils and monocytes (macrophages), and is induced by cytokines such as IFN-γ, IL-1β, and TNF-α [22,23]. Despite the higher expression levels of LIX, no significant differences were observed in the neutrophil count in mice irradiated with 0.1 Gy when compared to control mice. It is not clear why the expression levels of LIX were higher in the 0.1 Gy group but not in the 1 Gy group. The dose rate and fraction of radiation, or the radiation source might have attributed to our findings, and further studies are necessary to clarify this observation.

Our study has several limitations. First, we used a single fraction of 0.1 and 1 Gy X-ray radiation. Considering the effects of chronic and low-dose radiation on health, using a much lower dose rate and multiple radiation fractions would be desirable to evaluate their biological effect. Second, despite the use of a sham control, the small study sample size might have caused variations in the study results.

In conclusion, we observed that a single fraction of low or moderate dose of X-ray radiation does not cause significant changes in the immune system of C57BL/6 mice.
Unexpectedly, mice that received a low dose of X-ray radiation had a higher proportion of CD3⁺ T-cells and higher expression levels of LIX compared to mice that received a moderate dose of radiation. Since our study involved a small number of animals using single-fraction X-ray irradiation, further studies are necessary to elucidate the mechanism underlying our results.

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