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Radiation-induced Alterations in Immune Response to *Staphylococcus aureus* Infection

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1. Introduction

In an age when medical advances and terrorist threats frequently make news headlines, exposure to radiation is quickly becoming an issue of public, private, and government interest. Radiotherapy is a common treatment modality for cancer and other diseases. However, there are also equally clear hazards, such as the use of radioactive materials in acts of terrorism or war. Concomitant accidental or terrorism-related exposure to sublethal gamma or mixed-field (gamma and neutron) radiation would inevitably increase morbidity among individuals exposed to microbes. Ionizing radiation damages the haematopoietic and gastrointestinal systems. Prompt, sublethal irradiation increases the susceptibility to bacterial infections by decreasing the number of circulating mature white blood cells in the intestine (1).

The data presented herein represent the first results exploring the effects of whole-body irradiation on the ability of the immune system to respond to microbes. We utilized γ -ray radiation as a model for radiation exposure and then challenged the animals 4 days post-exposure to investigate the immune response in the most vulnerable phase of the hematopoietic-immune system. We employed *Stapylococcus aureus* bacterial challenges, a Gram-positive bacterium that is a major cause of septic shock and death.

2. Materials and Methods

2.1 Animals

Female mice (Balb/c, 6-7 weeks old) were purchased from Samtaco Animal Laboratory Co. (Daeheon, Korea). Animals were acclimatized for 1 week before irradiation and maintained in a room controlled for temperature, humidity, and a 12:12 hr light:dark cycle. Food and water were provided ad libitum. Female mice were used to minimize aggressive behavior and to compare with immune studies conducted previously in our laboratories. This study was approved by the Institutional Animal Care and Use Committee.

2.2 Bacteria

S. aureus 25923, acquired from ATCC, was cultured at 37℃ in Trypticase soy broth (Difco, Detroit, MI, USA), harvested at the mid-logarithmic growth phase, washed twice, and resuspended in PBS. The concentration of resuspended bacteria was determined spectrophotometrically at 620nm. Mice were injected *i.p*. with bacterial suspension containing 1.5×10^7 CFU live S. aureus.

2.3 Irradiation

The mice were randomized, placed in ventilated Plexiglas containers, and exposed to gamma radiation from the ^{60}Co Theratron-780 (Atomic Energy of Canada, Ltd., Canada) at a dose of 3 Gy (2.04 Gy/min).

2.4 Blood and Spleen Collection and Processing

Whole blood was collected in [K_2]EDTA-containing syringes by eye and spleen leukocytes were processed into single-celled suspensions. Hemologic monitoring was conducted 24 h after irradiation by a Sistema XE2100-D.

2.5 Spontaneous and Mitogen-induced In Vitro Blastogenesis

Splenic leukocytes were dispensed into microtiter plates (0.1 ml/well) followed by addition of 0.1 ml of medium containing Con A, LPS or medium without any mitogens. The proliferating cells were determined immediately (spontaneous blastogenesis) or 48 h after incubation period (mitogen-induced blastogenesis) with Cell Counting Kit-8 according to the manufacturer's protocol.

3. Results

3.1 Changes of blood cell populations

To study the effect of S. aureus infection on sublethally irradiated mice, we first examined the number of blood cells 24 h after bacterial challenge. The number of white blood cells (WBC) and platelet decreased from 7.620 ± 0.217 to $1.577 \pm 0.056\times10^3/\mu L$,

from 699.3 ± 41.6 to 614.7 ± 10.2×10^3/μL, respectively, in irradiated mice. The S. aureus challenge on irradiated mice did not change the number of WBC whereas the number of platelet was more decreased than irradiated mice to 501.0 ± 12.4×10^3/μL. As expected, there were significant changes in subpopulation of WBC such as lymphocytes and neutrophils in irradiated mice. However, the effect of bacterial challenge was only found in the number of neutorpils, approximately 74 % and 55 % decrease compared to the control and irradiated mice. In the case of RBC, the variation of total number was not great but the incidence of decrease was statistically clear.

3.2 Immune responses of splenic leukocytes

On the 5th day after sublethal dose of irradiation, the number of spleen cells in irradiated mice was only 16.5% of that of untreated control mice, and it slightly increased to 18.8% in bacterial infected mice. Despite of the previous reports that exposure to radiation leads to an acute increase of spontaneous blastogenesis, there were no remarkable effects on individual groups in this study. In contrast, mitogen-induced blastogenesis were found in radiated groups while the bacterial infected mice were decreased that responses, suggesting that the radiation caused some alteration in immune response may be closely related with the activity of immune cells not the numbers of them.

4. Conclusion

While bacterial infections are typically resolved by innate immune mechanisms, chronic infections will necessarily involve lymphocyte subpopulations (2). Sepsis is already known to lead to an increase in lymphocyte apoptosis (4), and subsequently, most sepsis patients are lymphopenic (5). Further dysregulation of these populations by radiation could severely limit the ability to respond to chronic bacterial infections.

In this study, these data demonstrated that the ability to respond to a mitogen was diminished by radiation following Gram-positive bacterial challenge. Acutely, neutrophils that normally traffic to the site of infection at the first line of defense against pathogens was decreased by irradiation, and even more decrease were shown in infected animals. However, the total number of spleen cells and peripheral WBC were not significantly changed in irradiated mice with or without bacterial challenge.

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REFERENCES

[1] Alper T, Cellular Radiobiology. New York: Cambridge University Press, 1979.
[2] Cohen J, The immunopathogenesis of sepsis, Nature, 420, 885-891, 2002.
[3] Bone RC, Gram-positive organisms and sepsis, Arch Intern Med, 154, 26-34, 1994.
[4] Tsiotou AG, Sakorafas GH, Anagnostopoulos G and Bramis J, Septic shock; current pathogenetic concepts from a clinical perspective, Med Sci Monit 11, RA76-85, 2005.
[5] Hotchkiss RS, Tinsley KW, Swanson PE, Schmieg RE, Hui JJ, et al. Sepsis-induced apoptosis causes progressive profound depletion of B and CD4+ T lymphocytes in humans, J Immunol, 166, 6952-6963, 2001.