Effect of Ionizing Radiation on the Host Resistance Against *Listeria Monocytogenes* Infection and the Cytokine Production in Mice

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Purpose : To evaluate the qualitative immunologic changes by ionizing radiation, we studied the altered capacities of the macrophages and lymphocytes to produce cytokines in conjunction with resistance to *Listeria monocytogenes* (LM) infection in mice.

Materials and Methods : BALB/c mice and *Listeria monocytogenes* were used. The mice were infected intraperitoneally with 10^5LM at 1 day after irradiation (300cGy) and sacrificed at 1, 3, 5 days after infection, and then the numbers of viable LM per spleen in the irradiated and control group were counted. Tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), interleukin-2 (IL-2), and nitric oxide (NO) were assessed after irradiation.

Results : Under gamma-ray irradiation with a dose range of 100-850cGy, the number of total splenocytes decreased markedly in a dose-dependent manner, while peritoneal macrophages did so slightly. Cultured peritoneal macrophages produced more TNF-α in the presence of lipopolysaccharide (LPS) during the 24 hours after *in vitro* irradiation, but their capacity of TNF-α production showed a decreased tendency at 5 days after *in vivo* total body irradiation. With 100cGy and 300cGy irradiation, cultured peritoneal macrophages produced more NO in the presence of LPS during the 24 hours after *in vitro* irradiation than without irradiation. Activated splenocytes from irradiated mice (300cGy) exhibited a decreased capacity to produce IL-2 and IFN-γ with Concavalin-A stimulation at 3 days after irradiation. When BALB/c mice were irradiated to the total body with a dose of 300cGy, they showed enhanced resistance during early innate phase, but a significant inhibition of resistance to LM was found in the late innate and acquired T-cell dependent phases.

Conclusion : These results suggest that increased early innate and decreased late innate and acquired immunity to LM infection by ionizing radiation

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(300cGy) may be related to the biphasic altered capacity of the macrophages to produce TNF-α and the decreased capacities of the lymphocytes to produce IL-2 and IFN-γ in addition to a marked decrease in the total number of cells.

Key Words : Radiation, *Listeria monocytogenes* infection, Host resistance, Cytokines

INTRODUCTION

Intracellular bacterial and viral infections are the main problems in the immunocompromised hosts. Intracellular bacterial pathogens and viruses are phagocytosed into the cell immediately after infection, proliferate within the cell and are transmitted intercellularly. So in these conditions they are not easily treated by antibiotics and are not controlled by humoral immunities with antibodies[1, 2]. The host resistance to them depends on the cell-mediated immunity[1-4]. Among intracellular bacterial infections, experimental murine listeriosis offers a useful model for the study of nonspecific and acquired T-cell mediated specific resistance to infection with facultative intracellular bacteria[5, 6]. The response to *Listeria monocytogenes* (LM) consists of two parts : a primary nonspecific phase and a later, antigen-specific T cell-dependent one[4]. Cytokines such as tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ) and interleukin-2 (IL-2) produced by immunocompetent cells are known to play a crucial role in the resistance to LM infection[7-9].

It is well known that total body irradiation reduces the total number of lymphoid cells and induces immunosuppression[10]. To understand more about the qualitative immunologic changes by ionizing radiation, altered capacities of the macrophages and the lymphocytes to produce cytokines were studied, in conjunction with the resistance to LM infection after irradiation in mice.

MATERIALS AND METHODS

1. Animals

BALB/c mice, 8-12 weeks old, from KAIST (Korea Advanced Institute of Science and Technology) were used and grouped under six per cage sized 18×28cm. All the procedures involving animals were approved by the institutional animal care and use committee.

2. Bacteria

Listeria monocytogenes (strain 10403 serotype 1) provided by Dr. D.K. Bishop (University of Utah, Salt Lake City, UT) has been maintained in a virulent state by repeated passage in mice. Bacteria were grown in brain heart infusion (BHI) medium.

3. Irradiation

The mice were placed in a container made out of wood and acryl and were given total body irradiation (TBI) at a dose rate of 80cGy/min using Cobalt-60 teletherapy unit. For *in vitro* irradiation of cells the field size was determined according to the size of the culture plates.

4. Cell culture

L929 cells for the TNF-α and IFN-γ assay, CTLL-2 cells for the IL-2 assay and macrophages were cultured in RPMI 1640 (Gibco Laboratories, Grand Island, NY) supplemented with 10% fetal calf serum (FCS), antibiotics, L-glutamine, 2-mercaptoethanol. J774 cells were cultured with Dulbecco's Modified Eagle's medium (DMEM) (Gibco Laboratories, Grand Island, NY).

5. Infection

One day after irradiation, the control group and the irradiated group of mice received a intraperitoneal (i.p.) injection of 10^5LM contained in a total of 1ml phosphate-buffered saline (PBS). Mice were sacrificed at 1, 3, 5 days after infection and spleens were aseptically removed and homogenized (Ultra-Turrax, Tekmar Co., Cincinnati, OH)

in PBS containing 0.05% Triton X-100. The splenic homogenates were quantified on the basis of their ability to form colonies on trypticase soy agar. Colony counts were determined after incubating the plates for 48 hours in a 5% CO_2 incubator.

6. Isolation of peritoneal macrophages

Peritoneal macrophages were obtained from BALB/c mice, as previously described by Beller et al[11]. Peritoneal exudate cells were harvested in Hanks balanced salt solution (HBSS) containing 1% FCS and cultured with RPMI 1640 medium. Cells were allowed to adhere at 37℃ for 3 hours and rinsed with PBS, and then adherent cells were incubated with 2.5mM sodium pyrophosphate solution and removed with a rubber policeman. Monolayers of resident macrophages were plated on 24 well-culture dishes (Costar Inc., Cambridge, MA) at a concentration of 1×10^6 cells per well and cultured for 24 hours.

7. IFN-γ assay

To observe the effect of ionizing radiation on the IFN-γ production by murine splenocytes, the mice were irradiated (100-600cGy) and the unirradiated group of mice were saved as the normal control. Three days after irradiation, mice were sacrificed and their splenocytes were rinced twice with HBSS and cultured in a 24-well plate at a concentration of 1×10^7 cells per well with RPMI 1640 medium in the presence of Concavalin-A (Con-A) 5μg/ml. The 24-hour culture supernatants were assayed for the IFN-γ by the L929 protection assay from encephalomyelocarditis (EMC) virus infection[12, 13]. The IFN-γ titers were defined as the reciprocal of the lowest dilution capable of protecting 50% of cells in staining.

8. IL-2 assay

A Group of BALB/c mice were irradiated (300 cGy). The unirradiated group of mice were saved as the normal control. At three days after irradiation, mice were sacrificed and their splenocytes were cultured at a concentration of 1×10^7 cells per well in RPMI 1640 medium in the presence of an optimum amount of Con-A. The 24-hour culture supernatants were harvested for assessment of IL-2 by using the method of Mosmann, et al[14]. The amount of IL-2 contained in cell supernatants was assessed by their ability to support the viability of CTLL-2 indicator cell line[14, 15]. Anti-IL-4 antibody (11B11) was used to block the action of IL-4.

9. TNF-α assay

To evaluate the effect of *in vitro* irradiation on TNF-α production by peritoneal macrophages, thioglycollate-elicited peritoneal macrophages were irradiated *in vitro* (100-850cGy) and then cultivated for 1 day in the absence and in the presence of 1μg lipopolysaccharide (LPS)/ml (*E. Coli* 0111:B4). TNF-α activity in each culture supernatant was assayed by the L929/Actinomycin D (Act D) cytotoxicity method.

To evaluate the effect of ionizing radiation, LPS and LM infection on TNF-α production *in vivo*, BALB/c mice (five mice per group) were given total body irradiation (300cGy). One day after irradiation, each group of animals received single i.p. injection of 10^5LM contained in a total of 0.5ml PBS. Five days after infection, mice were induced to produce TNF-α *in vivo* by intravenous injection of 25μg LPS/mouse in 0.2ml PBS. Two hours later, the animals were killed and the total blood was collected to get the serum. TNF-α activity in each serum sample was assayed by the L929/Act D cytotoxicity method.

To evaluate the effect of *in vivo* irradiation on TNF-α production by the peritoneal macrophages, BALB/c mice were irradiated *in vivo* (100 to 600 cGy). Two days later, the animals were injected intraperitoneally with thioglycollate broth and then peritoneal macrophages were harvested on the third day of injection. Thioglycollate-elicited peritoneal macrophages were cultivated for 1 day in the presence of 1μg LPS/ml. TNF-α activity in each culture supernatant was assayed by the L929/Act D cytotoxicity method.

TNF-α activity of the supernatant was assayed

by measuring their capacity to lyse L929 cell monolayers in the presence of Act D[12]. After incubation of L929 cells (10^4 cells/well) in 96-well plates at 37℃ for 18 hours, duplicate two fold dilutions of TNF-containing supernatants were mixed in 96-well microtiter plates with 10^4 L929 cells/well. Act D solution (2 μg/ml final concentration in RPMI 1640) was then added to each well. The viability of target cells was evaluated by the use of 3-[4,5-dimethylthiazole-2-yl]-2,5 diphenyl tetrazolium bromide (MTT). The formation of insoluble blue crystal by the cleavage of MTT by living cells was quantitated by reading the absorbance of each well at 570nm with an ELISA reader (Physica Co., New York, NY). The TNF titer of each supernatant was defined as the reciprocal of the lowest dilution capable of lysing 50% of the cells in the monolayer.

10. Nitric oxide (NO) measurement

Thioglycollate-elicited peritoneal macrophages were irradiated *in vitro* (100-850cGy) and then cultivated for 1 day in the absence and in the presence of 1 μg LPS/ml. NO was quantitated by the method using Griess reagent[16].

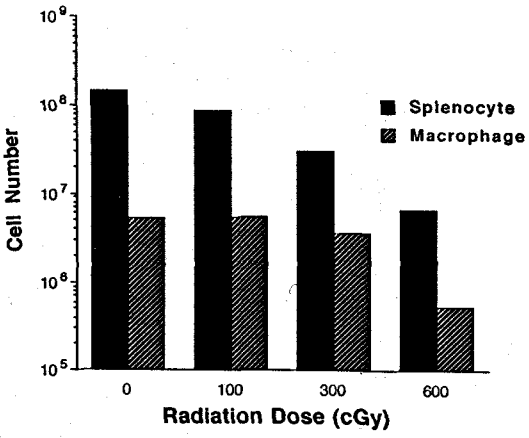

Fig. 1. Three days after irradiation, total number of splenocytes were counted. Total peritoneal macrophages were harvested three days after thioglycollate broth injection on the 2nd day after irradiation. Data represent the mean of duplicate experiments.

RESULTS

1. Effect of ionizing radiation on the number of splenocytes and peritoneal macrophages

At three days after total body irradiation of mice, the total number of splenocytes was 1.47×10^8 in the control group and 8.8×10^7, 3×10^7, 6.5×10^6 in the 100, 300 and 600cGy group, respectively (Fig. 1). So the number of splenocytes decreased markedly in proportional to the radiation dose. The total peritoneal macrophages were harvested at three days after thioglycollate broth injection which was done on the second day after irradiation. The number of peritoneal macrophages was 5.2×10^6 in control group and 5.4×10^6, 3.7×10^6, 5.3×10^5 in the 100, 300 and 600cGy group, respectively (Fig. 1). So there was no significant change after 100 and 300cGy but about 10-fold decrease was seen in the 600cGy group.

The effect of *in vitro* irradiation on the proliferation and viability of murine splenocytes was evaluated with doses from 100 to 2500cGy. Upon irradiation, the each group of cells was plated with two-fold serial dilution and cultivated in the Con-A-supplemented medium (5 μg/ml). The viability of total cells in each well was measured by MTT assay at 3 days after irradiation. Three days after irradiation the viability of splenocytes decreased in proportion to the radiation dose (Fig. 2). With the same method we observed the proliferation and viability of J774 macrophages after various doses of radiation from 100 to 2500cGy. Upon irradiation, each group of cells was plated at a concentration of 10^4 cells per well and the viability of total cells in each well was measured by MTT assay at 1 day and 3 days after irradiation. One day after irradiation no difference was observed between the irradiated and control groups. However at 3 days after irradiation the viability of J774 macrophage cells decreased in proportion to the radiation dose.

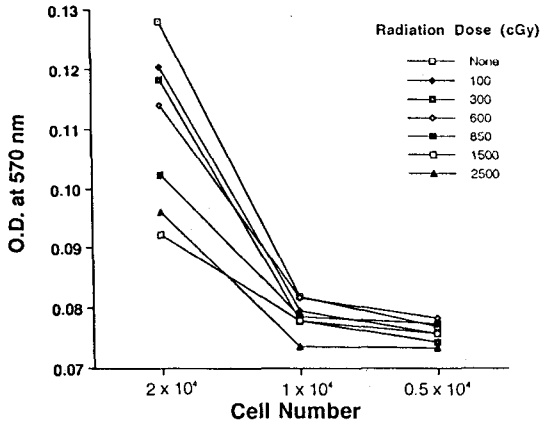

Fig. 2. Proliferation and viability of murine splenocytes after various doses of radiation (100 to 2500 cGy). Upon irradiation, each group of cells were plated with two-fold serial dilution and cultivated in the Con-A-supplemented medium (5 μg/ml). The viability of total cells in each well was measured by MTT assay 3 days after irradiation.

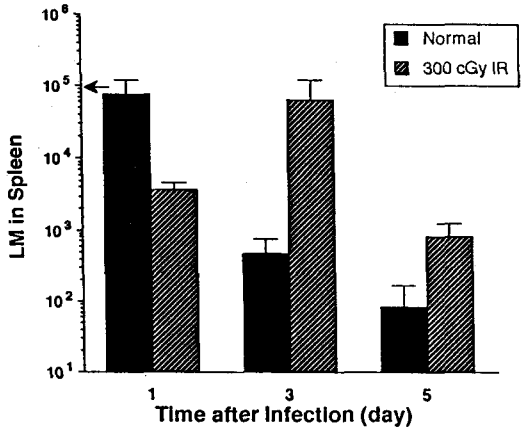

Fig. 3. Effect of ionizing radiation (300cGy) on the course of a primary sublethal LM infection. One day after irradiation, each group of animals received intraperitoneal injection of 105 LM contained in a total of 0.5 ml of normal saline. Mice were killed one and five days after infection or in a separate experiment, three days after infection. The number of viable LM per spleen was determined by plating on trypticase soy agar. Results expressed are the mean±SD from three mice per each time point.
← : Number of LM challenged.

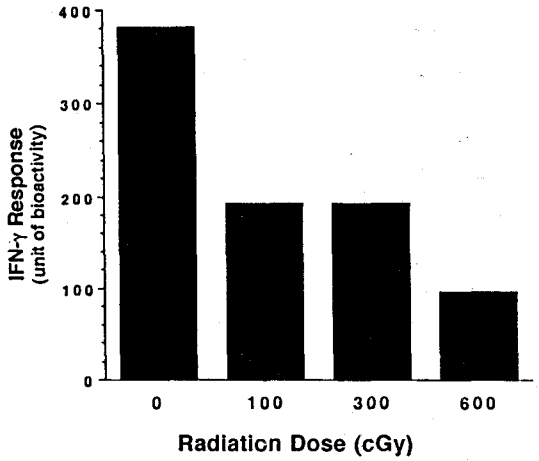

Fig. 4. IFN-γ production by murine splenocytes. Three days after irradiation, mice were sacrificed and their spleen cells were cultured at 1×10^7/ml in RPMI 1640 in the presence of optimum amount of Con-A. The 24-hour culture supernatants were assayed for IFN-γ by L929 protection assay from EMC virus infection. IFN-γ titers were defined as the reciprocal of the lowest dilution capable of protecting 50% of the cells in staining.

2. Effect of ionizing radiation on the course of a primary sublethal LM infection

The mice were infected intraperitoneally with 10^5 LM at 1 day after irradiation (300cGy) and sacrificed at 1, 3, 5 days after infection, and then the numbers of viable LM per spleen in the irradiated and control group were counted (Fig. 3). The number of LM at 1 day after infection in the control group was 21 times more than in irradiated group. The irradiated group had 144 times more LM than in the control group at 3 days after infection, and 10 times more LM than the control group at 5 days after infection.

3. Effect of ionizing radiation on the IFN-γ and IL-2 production by the splenocytes

The splenocytes from the unirradiated control group were compared with those from irradiated group for their capacity to produce IFN-γ and IL-2. Activated splenocytes from irradiated mice exhibited a reduced capacity to secrete IFN-γ at

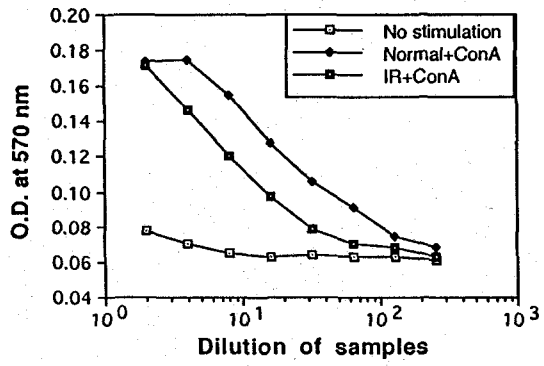

Fig. 5. IL-2 production by murine splenocytes. The unirradiated group of mice were saved as the normal control. Three days after irradiation (300cGy), mice were sacrificed and their spleen cells were cultured at 1×10^7/ml in RPMI 1640 in the presence of optimum amount of Con-A. The 24-hour culture supernatants were harvested for assessment of IL-2.

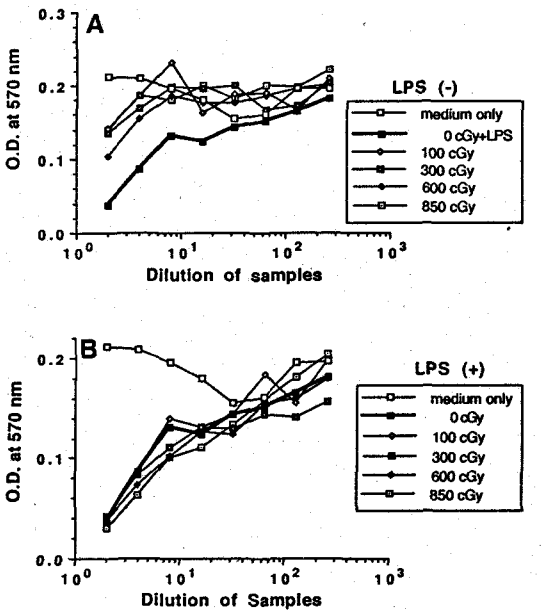

Fig. 6. TNF-α production by peritoneal macrophages. Thioglycollate-elicited peritoneal macrophages were irradiated *in vitro* (100-850cGy) and then cultivated for 1 day in the absence (A) or in the presence (B) of 1 μg LPS/ml (*E. coli* 0111). TNF-α activity in each culture supernatant was assayed by the L929/Act D cytotoxicity method.

3 days after irradiation of 100, 300 and 600cGy. The effect was particularly prominent in the 600 cGy group where the irradiated group showed a decreased capacity to secrete IFN-γ by a factor of 4 (Fig. 4).

In addition, activated splenocytes from irradiated mice showed a reduced capacity to produce IL-2 at 3 days after irradiation of 300cGy (Fig. 5).

4. Effect of *in vitro* irradiation on TNF-α production by the peritoneal macrophages

Thioglycollated-elicited peritoneal macrophages were irradiated *in vitro* (100 to 850cGy) and then cultivated for 1 day in the absence and in the presence of 1 μg LPS/ml. TNF-α activity in each culture supernatant was assayed by the L929/Act D cytotoxicity method (Fig. 6). TNF-α activity was detected only in the presence of LPS during the 24 hours after *in vitro* irradiation. Cultured peritoneal macrophages in the irradiated group produced slightly more TNF-α than in the unirradiated control group (Fig. 6B).

5. Effect of ionizing radiation, LPS and LM infection on TNF-α production *in vivo*

During a sublethal murine infection with LM, TNF-α activity was detectable in neither sera nor spleen homogenates at any stage of infection when a bioassay with L929 cells was used. The peak of TNF-α production induced by LPS was shown late (day 5) in the infection when serum samples were collected at 2 hours after LPS injection[17]. One day after irradiation of 300cGy, each group of mice received a single i.p. injection of 10^5LM contained in a total of 0.5ml PBS. Five days after infection, TNF-α production was induced *in vivo* by intravenous injection of 25 μg LPS/mouse in 0.2ml PBS. Two hours later, the animals were killed and total blood collected to get serum. TNF-α activity in each serum sample was assayed by L929/Act D cytotoxicity method (Fig. 7). TNF-α production of peritoneal macrophages decreased in the presence of LPS after *in vivo* whole body irradiation. With LM infection only

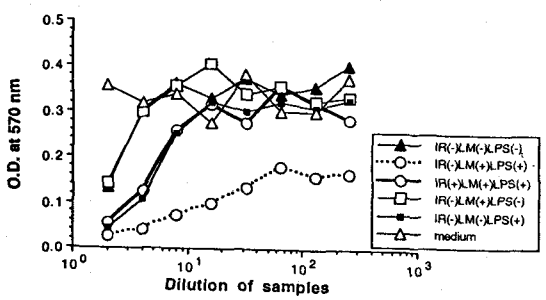

Fig. 7. Effect of ionizing radiation, LPS and LM infection on TNF-α production *in vivo*. One day after irradiation (300cGy), each group of animals received single i.p. injection of 10^5LM contained in a total of 0.5ml PBS. Five days after infection, mice were induced TNF-α production *in vivo* by injection of 25μg LPS/mouse in 0.2ml PBS. Two hours later, the animals were killed and collected total blood to get serum.

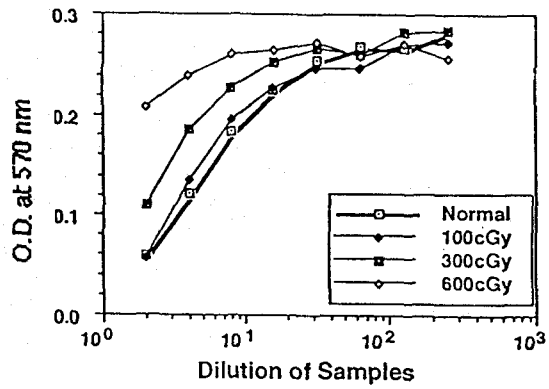

Fig. 8. TNF-α production by peritoneal macrophages. BALB/C mice were irradiated *in vivo* (100-600 cGy). Two days later, the animals were injected intraperitoneally with thioglycollate broth and then peritoneal macrophages were harvested on the third day of injection. Thioglycollate-elicited peritoneal macrophages were cultivated for 1 day in the presence of 1μg LPS/ml.

TNF-α activity was not detected and with LPS after LM infection TNF-α activity increased markedly. However with LPS and LM infection after irradiation the TNF-α activity in serum was lower than in the unirradiated group with LPS and LM infection.

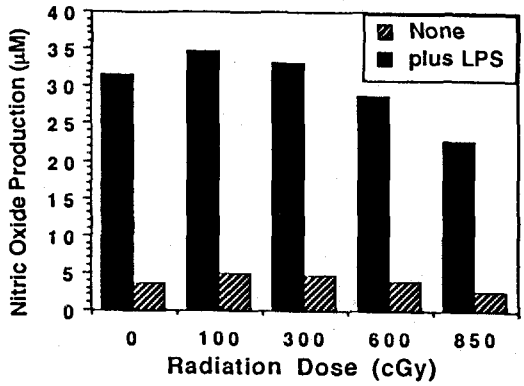

Fig. 9. Production of nitric oxide by peritoneal macrophages. Thioglycollate-elicited peritoneal macrophages were irradiated *in vitro* (100-850 cGy) and then cultivated for 1 day in the absence or in the presence of 1μg LPS/ml. Nitric oxide was quantitated by the method using Griess reagent.

6. Effect of *in vivo* irradiation on TNF-α production by peritoneal macrophages

Two days after irradiation of 100, 300 and 600 cGy, the irradiated and unirradiated mice were injected intraperitoneally with thioglycollate broth and then peritoneal macrophages were harvested on the third day of injection. Thioglycollate-elicited peritoneal macrophages were cultivated for 1 day in the presence of 1μg LPS/ml. TNF-α activity in each culture supernatant was assayed by the L929/Act D cytotoxicity method (Fig. 8). The capacity of TNF-α production in macrophages decreased in proportion to the radiation dose.

7. Effect of *in vitro* irradiation on the production of NO by peritoneal macrophages

Thioglycollate-elicited peritoneal macrophages were irradiated *in vitro* (100 to 850cGy) and then cultivated for 1 day in the absence and in the presence of 1μg LPS/ml. NO was quantitated by the method using Griess reagent (Fig. 9). NO production was not induced without LPS. With low dose irradiation (100 and 300cGy) cultured peritoneal macrophages produced more NO in the presence of LPS during the 24 hours after *in vitro* irradiation than without irradiation and less NO at the higher doses of 600cGy and 850cGy.

DISCUSSION

Most investigations dealing with the effect of radiation on the immunity had focused on the initiation of the primary antibody response[18,19]. Before 1960 the idea of cell-mediated immunity was a hypothesis rather than a fact. The importance of cell-mediated immune mechanisms was realized when it was established that foreign bone marrow cells injected into lethally irradiated recipients produced a wasting disease, termed secondary disease. In recent years, however, the realization that cell-mediated immunity plays an important role in the control of cancer, may be a potential therapy against cancer and, in fact , may be a determining factor in acquisition of cancer, has led to an increased interest in the effects of immunosuppressive agents, including radiation, on immunity[20]. Secondary immunosuppression increases the incidence of malignant tumors. This is closely related with the aging process and the infection which is the most serious complication in the immunocompromised host and frequently creates many problems after radiation therapy. Ionizing radiation increases the recipient's susceptibility to local and systemic infection by endogenous and exogenous microorganisms. There have been some reports about *K. pneumoniae* infection, tuberculosis, dermatophytosis or bacterial endocarditis in irradiated hosts[21-25].

Recently the studies about the cytokines have been introduced, so it has become possible to know the factors and mechanisms of immunologic changes and the method to modulate the immune response[26]. Investigations about the protection and therapy of radiation injury by the immunomodulators was started in 1985 and it has been reported that IL-1, IL-6, granulocyte-macrophage colony stimulating factor (GM-CSF), thrombopoietin, and other biologic response modifiers showed the radioprotection and therapy of radiation injury [27-34]. Reported studies were concerned with the cytokine effects on the survival prolongation and promoted hematopoiesis in the radiation-compromised animals. Peterson et al.[30] reported that biologic response modifiers that sustained and/or enhanced irradiation (7Gy)-induced expression of specific cytokine genes improved survival after experimental infection with *Klebsiella pneumoniae*. However there are few reports about the effects of irradiation on the production of cytokines[26]. The kinetics of the cytokine response to irradiation related to intracellular bacterial infection are poorly defined. So the purpose of this study is to investigate the qualitative immunologic changes by the ionizing radiation, that is, the altered capacities of the macrophages and the lymphocytes to produce the cytokines and the resistance to LM infection after the ionizing radiation.

The study by Tartakovsky et al.[35] demonstrated that low-dose irradiation (3 and 4Gy) induced production of IL-6 and macrophage-colony-stimulating factor in mice. In the study by Girinsky et al.[36] a increase in the levels of IL-6 and TNF in blood was shown in patients who had TBI prior to bone marrow transplantation. Also Xun et al.[37] reported that TNF-α appeared in the serum and colon 4 hours post-TBI and peaked in 24 hours, followed by increasing IL-1 alpha and then IL-6 levels. And TNF-α and IL-1 alpha decreased rapidly within 3 to 5 days post-TBI if no allogenic cells were transplanted. They propose that the interaction of inflammatory cytokines released by conditioning with histoincompatible donor cells is a critical process in the pathogenesis of acute graft-versus-host disease.

Experimental murine listeriosis offers a useful model for the study of nonspecific and acquired T cell mediated specific resistance to the infection with the facultative intracellular bacteria. The response to LM consists of two parts: a primary nonspecific phase and a later, antigen-specific T-cell-dependent one[4]. The primary response, which occurs during the first 48 hours after the onset of infection, has been attributed to the resident macrophages and the early influx of bone marrow-derived phagocytes in the liver and spleen[1]. These cells are not activated, but they do inhibit the growth of LM. Neither splenocytes nor

purified T cells from LM-infected mice will transfer the immunity to the naive animals during this time, implying that a T-cell-independent mechanism is responsible for this effector activity[1, 4]. The second response, beginning at about day 4, is characterized by the proliferation of antigen-dependent T cells which further enhance the bacterial killing *in vivo*[1]. These cells can adoptively transfer the immunity in an immunogenetically restricted manner to the naive recipient mice. Studies from a number of laboratories have established the crucial role of cytokines produced by LM-dependent T-cells in enhancing the bactericidal capabilities of phagocytes during the second phase[8], and it has been suggested that macrophage activation resulting from the release of these T cell products may account for the ultimate resolution of the infection[1, 8].

In our study the number of LM at 1 day after sublethal LM infection in the 300cGy-irradiated group was 21 times less than in the control group, but 144 times more than in the control group at 3 days after irradiation and 10 times more than in the control group at 5 days after irradiation. So the present study demonstrated that when mice were irradiated to the total body with a dose of 300cGy, they showed the enhanced resistance during the early innate phase but a significant inhibition of the resistance to LM in the late innate & acquired T-cell dependent phases.

We suggest this result is associated with not only a markedly decreased number of lymphocytes but also qualitative changes in the immunocompetent cells.

The evidence for a role of TNF-α during early stages of infection can be extended to our results gained from the examination of the effects of exogenous LPS and irradiation on TNF-α production by the peritoneal macrophages and on the resistance to listeriosis. A plausible function for TNF-α in anti-Listeria resistance is the recruitment of blood monocytes into the infection foci. In this regard, it is known that TNF-α induces IL-1 synthesis in a variety of cell types. Both of these cytokines are potent mediators of the inflammation, elicit the production of the chemotactic factors, and cause the alterations in the vascular endothelium. Taken together, it has been suggested that TNF-α and/or IL-1 induced by LPS could attract more inflammatory cells having anti-Listeria function into the sites of infection, although it might be possible that the increase of the blood leukocytes influenced the peritoneal exudate cells.

In our study, in the presence of LPS during the 24 hours after *in vitro* irradiation the irradiated cultured peritoneal macrophages produced slightly more TNF-α than unirradiated control group. When thioglycollate-elicited peritoneal macrophages were cultivated for 24 hours in the presence of LPS at 5 days after irradiation, the capacity of TNF-α production in the macrophages decreased in proportion to the radiation dose. Also in the experiment concerning the effect of ionizing radiation, LPS and LM infection on TNF-α production *in vivo*, TNF-α activity in the serum decreased in the presence of LPS at 5 days after LM infection in the irradiated group. The TNF-α activities differed between 1 day after *in vitro* irradiation and at 5 days after *in vivo* irradiation. As a result we evaluated the effect of *in vitro* irradiation on the production of NO by the peritoneal macrophages. Thioglycollate-elicited peritoneal macrophages were irradiated *in vitro* and then cultivated for 1 day in the absence and in the presence of LPS. With low dose irradiation (100 and 300cGy) the cultured macrophages produced more NO in the presence of LPS during the 24 hours after *in vitro* irradiation, and we suggest the increased NO production is related to the increased TNF-α activity at 1 day after *in vitro* irradiation of peritoneal macrophages. Also we suggest the increased TNF-α activity of the peritoneal macrophages at 1 day after *in vitro* irradiation is related to the decreased number of LM at 1 day after sublethal LM infection in the 300cGy-irradiated group, and the enhanced resistance to infection during the early innate phase.

However at 3 days after the infection, the irradiated group had 144 times more LM than the control group, and at 5 days after the infection 10

times more LM than the control group. Among T-cell products, participation of IFN-γ in the antilisterial resistance has been well characterized. Although IFN-γ production is below the detection limit in the sera of LM-infected mice at all stages of infection, Nakane[17] reported that significant IFN-γ production could be induced in the bloodstream in response to the specific antigen late in the infection. IL-2 induces T-cell proliferation in an autocrine manner and provides a means by which antigen-triggered T cells can be clonally expanded *in vitro* and also induces the cytokine production in T-cell. We observed that activated splenocytes from the irradiated mice showed a reduced capacity to produce IFN-γ and IL-2 at 3 days after the irradiation of 300cGy in the presence of an optimum amount of Con-A. We suggest decreased acquired immunity to LM infection by the ionizing radiation (300cGy) may be related to the reduced capacities of lymphocytes to produce IFN-γ and IL-2.

In conclusion, our present study suggests that the increased early innate and decreased late innate and acquired immunity to LM infection by the ionizing radiation may be related to the biphasic altered capacity of the macrophages to produce TNF-α and the decreased capacities of the lymphocytes to produce IL-2 and IFN-γ, in addition to a marked decrease in total cell numbers.

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= 국문 초록 =

방사선조사후 마우스에서의 Cytokine 생산능 및 *Listeria monocytogenes*에 대한 저항성의 변화

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목 적 : 방사선조사가 세포내재성 병원균인 *Listeria monocytogenes* (LM)의 감염에 미치는 영향과 함께 감염면역과 밀접한 관계가 있는 대식세포에서의 TNF-α 및 Nitric oxide (NO) 생산능의 변화와 비장세포에서의 IFN-γ 및 IL-2생산능에 미치는 영향을 알아보고자 본 실험을 시행하였다.

대상 및 방법 : 실험동물로는 BALB/c 마우스를 사용하였으며 Co-60 원격치료기를 이용하여 방사선을 조사하였다. LM감염에 미치는 영향을 관찰하기 위해서 방사선조사 1일후 10^5의 LM균을 복강내에 주사하고 1일, 3일, 5일후에 비장조직에서 LM생균수를 측정하였다. 방사선조사가 마우스의 생체내에서와 시험관내에서의 TNF-α의 생산능에 미치는 효과를 관찰하기 위해서 각각 LPS로 유도하여 L929/Actinomycin D assay에 의해 TNF-α량을 측정하였다. IFN-γ의 생성능은 방사선조사후 비장을 적출하여 비장세포액을 제조하여 Concavalin A (Con-A)로 자극한 후 정량검사를 하였다. IL-2의 생성능은 IFN-γ실험에서와 같이 비장세포를 얻어서 Con-A로 자극하여 CTLL-2세포의 성장촉진능력을 관찰함으로써 판정하였다. 방사선조사가 복강내 대식세포에서 생산되는 NO에 미치는 영향도 관찰하였다.

결 과 : 방사선 (300cGy)을 조사한 군에 LM을 감염시킨 1일후 비장으로부터 검출되는 생균수는 대조군에 비해 감소하였으나 감염 3일 5일후의 생균수는 대조군에 비하여 오히려 증가하였다. 시험관내 복강대식세포에 방사선 (100-850cGy)을 조사하면 1일후 생산되는 TNF-α의 양은 대조군에 비하여 증가하였으나, 방사선조사 (100-600cGy) 5일후 수집된 복강대식세포에 의한 TNF-α의 생산은 오히려 감소하였고, 방사선조사 (300cGy)후 LM감염시 5일후 유도된 생체내 TNF-α의 생산도 대조군에 비해 감소하였다. 방사선 (300cGy)을 조사한 마우스로부터 적출한 비장세포로부터 생산되는 IFN-γ와 IL-2의 양은 대조군에 비해 감소하였다. NO의 양은 100cGy 및 300 cGy조사시 대조군에 비해 증가하였으며 그이상 조사량을 증가하면 점차 감소하였다.

결 론 : 방사선조사후 세포내재성 병원균인 LM감염에 대한 초기 저항성의 증가와 감염중반이후의 저항성 감소는 대식세포에서의 초기 TNF-α 생산능 증가후 감소, 그리고 T림프구에서의 IFN-γ 및 IL-2 생산능 감소와 관련이 있을 것으로 사료된다.