A single low-dose irradiation with X-rays stimulates NK cells and macrophages to release factors related to the cytotoxic functions of these cells

EWA M. NOWOSIELSKA, JOLANTA WREMBEL-WARGOCKA, ANETA CHEDA, MAREK K. JANIAK

Department of Radiobiology and Radiation Protection, Military Institute of Hygiene and Epidemiology in Warsaw, Poland

Abstract

The results of our previous study indicate that stimulated natural killer (NK) lymphocytes and cytotoxic macrophages are involved in retardation of the development of syngeneic tumour nodules in mice pre-exposed to low-level irradiations with X-rays. In the present investigation we examined the selected parameters of these cells activity that may be responsible for the X-ray-induced enhancement of the NK cell- and macrophage-mediated cytotoxicity. BALB/c mice were whole-body irradiated (WBI) with a single dose of 0.1, 0.2, or 1.0 Gy of X-rays. Cytotoxic activities of NK cells and macrophages were estimated in vitro using the classical ^{51}Cr-release and [^{3}H] thymidine-uptake assays, respectively. Colorimetric assay with the Griess reagent was used for estimation of the nitric oxide (NO) synthesis. Surface expression of the Fas ligand (FasL) on NK splenocytes was determined using the anti-FasL antibody. The levels of interferon-γ (IFN-γ) and tumour necrosis factor-α (TNF-α) were examined in the incubation media of the collected NK cells and macrophages, respectively, using the ELISA assays. The results indicate that the low-level radiation-induced enhanced cytotoxicity of the two cell types coincided with the elevated expression of FasL on the NK lymphocytes and the increased production of NO and TNF-α in the activated macrophages.

Key words: *macrophages, NK cells, low-doses of X-rays, cytotoxicity, NO*

(Centr Eur J Immunol 2006; 31 (1-2): 51-56)

Introduction

Experimental evidence from the recent years indicates that low-level irradiations with X- or γ-rays may inhibit the development of both primary and secondary tumours [1-3]. In our previous study we demonstrated that whole-body irradiation of mice with a single low (0.1 and 0.2 Gy) but not higher (1.0 Gy) dose of X-rays led to a significant retardation of the development of pulmonary tumour nodules induced by the intravenous injection of syngeneic L1 sarcoma cells and that the effect was related to the enhanced cytotoxicity of natural killer (NK) cells and/or activated macrophages [4, 5].

Anti-tumour functions of NK lymphocytes and macrophages rely on the non-specific recognition of the target cells followed by the enhanced expression and/or secretion of the selected "killing-related" ligands and cytokines. Thus, stimulated NK cells exhibit elevated amounts of a ligand responsible for aggregation of the Fas molecules on the surface of the target cells and initiating apoptotic death of the latter [6-11]. In turn, activated macrophages produce nitric oxide (NO) and tumour necrosis factor-α (TNF-α) – the two related factors involved in the induction of apoptosis in the target cells [12-15].

In view of this, the aim of the present study was to asses the expression of FasL by NK cells as well as the synthesis of NO and TNF-α by macrophages obtained from mice pre-exposed to low (0.1 or 0.2 Gy) versus higher (1.0 Gy) doses of X-rays.

Correspondence: Ewa Nowosielska, Department of Radiobiology and Radiation Protection, Military Institute of Hygiene and Epidemiology in Warsaw, Poland, e-mail: ewan14@wp.pl, phone +48 22 681 61 35

Material and methods

Animals and irradiation

For the experiments, male, 6-8 week-old BALB/c mice obtained from the Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland, were used. The animals were whole-body irradiated (WBI) from the HS320 Pantak X-ray generator (230 kV, 20 mA) supplied with the 1-mm Al and Cu filters, at 2.2 Gy/h dose rate to obtain the absorbed doses of 0.1, 0.2 or 1.0 Gy per mouse (the absorbed doses were verified using thermoluminescent dosimeters implanted subcutaneously (s.c.) in the middle abdominal region). Control mice were sham-exposed (the generator at the off-mode) in identical conditions. All the studies were carried out by permission of the Local Ethical Committee for Experimentation on Animals at the National Institute of Public Health in Warsaw.

Tumour cells

L1 sarcoma cells were obtained from the Maria Skłodowska-Curie Memorial Cancer Centre and Institute of Oncology, Warsaw, Poland. These cells developed spontaneously in the lung of a BALB/c mouse [16]. YAC-1, a murine lymphoma cell line, was obtained from the Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland. P815 mastocytoma cells were obtained from Department of Microbiology, Medical University of Warsaw, Poland. The cells were grown in a culture medium (CM) composed of the RPMI-1640 medium (Sigma, Poznan, Poland), 10% FBS (GIBCO BRL, Karlsruhe, Germany), 100 U/ml penicillin, 100 µg/ml streptomycin (Polfa, Warsaw, Poland) and 2 mM L-glutamine (Sigma), and stabilized with Na_2CO_3 (Sigma).

Preparation of the NK cell suspension

NK cells were purified as previously described [17]. Briefly, single cell-suspensions in CM were prepared from the spleens of both irradiated and sham-irradiated mice and incubated on glass Petri dishes for 40 min. at 37°C in a humidified atmosphere of 95% air and 5% CO_2 (standard conditions); in each case the cells were collected and pulled from at least three mice. Non-adherent cells were then collected, washed, and incubated in the ammonium chloride solution for 30 s at room temperature (to lyse the erythrocytes). After washing and resuspending in CM the cells were passed through a nylon wool column and the wool-nonadherent cells were used for the NK cell-mediated cytotoxicity assay and the flow cytometric analysis.

Preparation of the macrophage-enriched cell suspensions

Mice were intraperitoneally (i.p.) injected with 10% Sephadex G-25 (Pharmacia, Uppsala, Sweden) and two days later peritoneal cells were pulled from at least four mice per each experimental group, resuspended in CM, and incubated on glass Petri dishes for 2 h in standard conditions. The glass-adherent cells were then harvested and resuspended in CM.

NK cell-mediated cytotoxicity assay

Cytotoxic activity of NK cells was measured on three consecutive days after the irradiation using the standard *in vitro* ^{51}Cr-release assay [18]. In brief, 10^6 YAC-1 target cells suspended in 0.1 ml CM were incubated for one hour in standard conditions with 5.55 MBq of $Na_2{}^{51}CrO_4$ (Polatom, Otwock-Swierk, Poland). After the incubation, the cells were washed with PBS and 100-µl aliquots containing 10^4 cells were placed in wells of a microtiter plate (Corning, Warsaw, Poland). The NK-enriched cell populations were then added at 100:1 effector-to-target (E:T) cell ratio; for each *in vitro* experimental group five replications were performed. After the four-hour incubation in standard conditions, aliquots of the cell-free supernatants were harvested and the radioactivity of ^{51}Cr released from the target cells was measured in a γ-counter (Auto-Gamma Cobra II gamma counter, Canberra-Packard, Warsaw, Poland). The rate of cytotoxic activity was calculated using the formula: 100% x [(experimental release – spontaneous release)/(maximum release – spontaneous release)]; the release of ^{51}Cr from the target cells cultured in the medium alone was taken as the spontaneous release, while ^{51}Cr released from the target cells lysed with 1% Trition X 100 (Sigma) was regarded as the maximum release.

Macrophage-mediated cytotoxicity assay

Cytotoxic activity of the macrophages was measured 2 h after the irradiation and then on the three consecutive days using the L1 sarcoma or P815 mastocytoma cells as targets [19]. Briefly, $4x10^6$ L1 or P815 cells were suspended in 2.5 ml CM supplemented with 0.3 MBq [^{3}H] thymidine and incubated for 20 h in standard conditions. The macrophage-enriched suspensions were then added at 20:1 E:T ratio with or without the addition of 50 U/ml IFN-γ (Sigma) and 100 ng/ml LPS (Sigma). After the 48-h incubation viable adherent cells were lysed, harvested, and their radioactivity was monitored in a β-counter (Tri-Carb 2100TR Counter, Canberra-Packard). The rate of the cytotoxicity was calculated using the formula: [(A – B)/A] x 100%, where A indicates isotope counts in the target cells cultured alone, and B indicates isotope counts in the target cells cultured with the effector cells.

Production of nitric oxide by peritoneal macrophages

Nitric oxide (NO) synthesized in the collected macrophages was quantitated 2 h after the irradiation and then on the three consecutive days based on the measurements of

the level of the NO_2^- anion [20]. Briefly, peritoneal macrophages were incubated in wells of a microtiter plate for 48 h in standard conditions with or without the addition of 50 U/ml IFN-γ (Sigma) and 100 ng/ml LPS (Sigma). After that, the supernatant was mixed with the same volume of the Griess reagent and kept for 10 min. in the dark at room temperature. Absorbance at 540 nm was then measured using the SpectraCount™ (Canberra Packard) microplate reader.

Production of IFN-γ and TNF-α

The NK-enriched cell suspensions collected on three consecutive days post-irradiation were incubated for 4 h in CM in standard conditions with YAC-1 target cells at E:T=100:1. After that, the supernatants were collected and used in the ELISA test (BD, Warsaw, Poland) for estimation of the IFN-γ production by NK cells. Peritoneal macrophages collected 2 h after the irradiation and on the three consecutive days thereafter were incubated for 48 h in CM in standard conditions with L1 as targets at E:T=20:1. After the incubation, the supernatants were collected and assayed for the level of TNF-α using the ELISA test (BD).

Flow cytometric analyses

NK-enriched spleen cells obtained from the sham-exposed and irradiated mice were stained with the PE-tagged antibody to the mouse Fas ligand (FasL; Santa Cruz Biotechnology, Santa Cruz, USA) to determine the surface expression of FasL. The estimation was carried out using the FACS Calibur flow cytometer equipped with the CellQuest software (BD).

Statistical analysis

Mann-Whitney U test for non-parametric trials was used for statistical analysis of the differences between the results obtained for each of the irradiated vs. sham-exposed groups and p values lower than 0.05 were regarded as significant.

Results

As indicated in figure 1, a single whole body exposure of mice to any of the three doses of X-rays significantly stimulated the cytotoxic activity of NK splenocytes, the effect being most pronounced on the second day after the irradiation. Interestingly, the WBI of mice with 1.0 Gy appeared to be a more potent stimulator of the NK cell-mediated cytotoxicity than exposures to either 0.1 or 0.2 Gy X-rays.

As shown in figure 2, NK lymphocytes obtained from mice 2 days after the single WBI with 0.1 and 0.2 Gy X-rays (i.e. at the time when the cytotoxic function of these cells was maximally stimulated) demonstrated the significantly increased surface expression of FasL as compared to the cells collected form the sham-exposed animals. No such effect was detected after the irradiation of mice with 1.0 Gy X-rays.

As shown in figure 3A, a single WBI of mice with 0.1 and, to a slightly higher extent, 0.2 Gy but not 1.0 Gy X-rays led to the significant stimulation of the cytolytic function of the IFN-γ- and LPS-boosted peritoneal macrophages against the P815 tumour cells. The stimulation was detectable already on the day of exposure, followed by a decrease on the first and second days, rising again on the

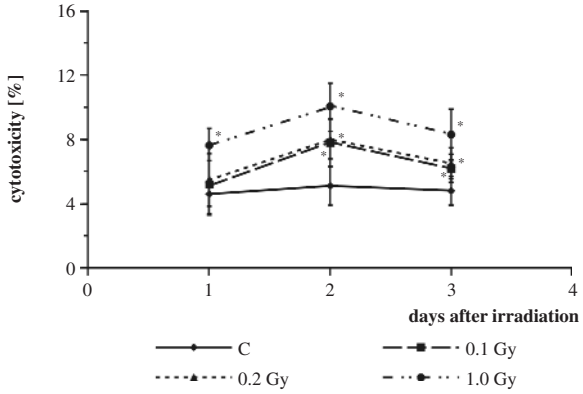

Fig. 1. Cytotoxic activity of splenic NK cells (E:T=100:1) on three consecutive days after irradiation of mice with 0.1, 0.2 or 1.0 Gy X-rays. C – sham-exposed (control) mice; 0.1 Gy – mice exposed to a single WBI with 0.1 Gy X-rays; 0.2 Gy – mice exposed to a single WBI with 0.2 Gy X-rays; 1.0 Gy – mice exposed to a single WBI with 1.0 Gy X-rays. Mean values±SD obtained from three independent experiments are presented; each experimental group consisted of three mice. *indicates statistically significant ($p<0.05$) difference from the control value

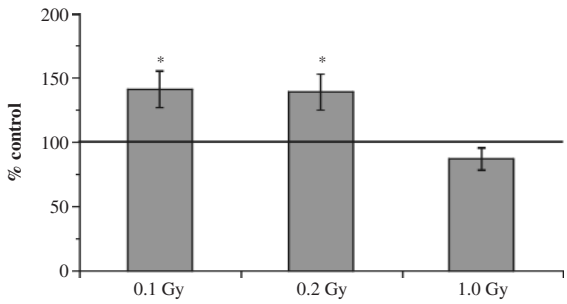

Fig. 2. Relative (percentage of the control value) surface expression of FasL on NK cells two days after a single WBI of mice with 0.1, 0.2 or 1.0 Gy X-rays. 0.1 Gy – mice exposed to a single WBI with 0.1 Gy X-rays and injected with L1 cells; 0.2 Gy – mice exposed to a single WBI with 0.2 Gy X-rays and injected with L1 cells; 1.0 Gy – mice exposed to a single WBI with 1.0 Gy X-rays and injected with L1 cells. Mean values±SD obtained from three independent experiments are presented; each experimental group consisted of three mice. *indicates statistically significant ($p<0.05$) difference from the control (100%) value

third day post-irradiation. Similar, although less pronounced effect, was detectable when the L1 tumour cells were used as targets in the cytotoxicity assay (Fig. 3B). Somewhat lower, but kinetically similar, stimulation of the cytotoxicity was exhibited against the two tumour targets when the effector macrophages were not incubated with IFN-γ and LPS (data not shown).

As indicated in figure 4, exposure of mice to either of the two low doses of X-rays significantly stimulated production of NO in the IFN-γ- and LPS-treated peritoneal macrophages. This effect was most pronounced as early as 2 h after the exposure and again on the 3rd day post-irradiation. In contrast, at the same time points macrophages obtained from mice irradiated with 1.0 Gy X-rays synthesized the markedly lower amounts of NO. Notably, none of the doses of X-rays used appeared to boost the synthesis of NO in macrophages incubated in the absence of IFN-γ and LPS in the CM (data not shown).

Figure 5 demonstrates that macrophages obtained from mice irradiated with all the three doses of X-rays and then cultured with L1 target cells secreted elevated amounts of TNF-α as early as 2 h post-exposure and then over the three consecutive days. However, the production of TNF-α measured on the 1st and 2nd days after irradiations with either of the two low doses was somewhat less pronounced than after the exposure to 1.0 Gy X-rays which resulted in the steadily elevated secretion of the cytokine throughout the whole period of observation.

Discussion

The results of the present study confirm our previous observation that 2 days after a single irradiation of mice with 0.1, 0.2 or 1.0 Gy X-rays the cytotoxic activity of NK lymphocytes obtained form these animals is significantly enhanced. Stimulation of the activity of NK cells by exposures to low-LET radiation was also reported by other authors. For example, Liu et al. [21] demonstrated that the WBI of mice with 0.075 or 0.5 Gy X-rays triggered the activity of NK cells tested 24 h post-exposure and Kojima et al. [7, 22] detected the enhanced activity of these cells 4 and 6 h after irradiation of mice with 0.5 Gy of γ-rays. Likewise, as reported by Ju et al. [23], increased cytolytic function of NK lymphocytes was detectable for six consecutive days

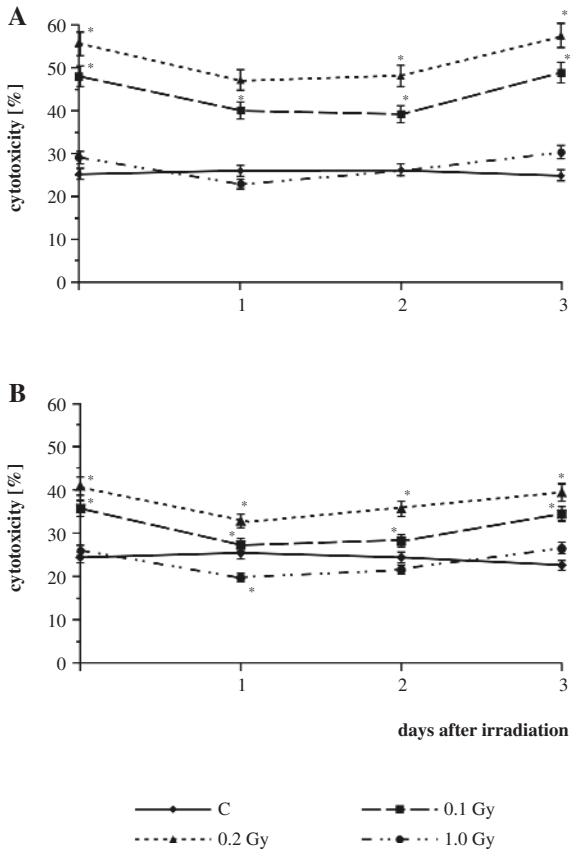

Fig. 3. Cytotoxic activity of the IFN-γ- and LPS-treated peritoneal macrophages on the day of WBI of mice with 0.1, 0.2 or 1.0 Gy X-rays (2 h post-irradiation) and on the three consecutive days against the P815 [A] and L1 [B] cells (E:T= 20:1). C – sham-exposed (control) mice; 0.1 Gy – mice exposed to a single WBI with 0.1 Gy X-rays; 0.2 Gy – mice exposed to a single WBI with 0.2 Gy X-rays; 1.0 Gy – mice exposed to a single WBI with 1.0 Gy X-rays. Mean values±SD obtained from three independent experiments are presented; each experimental group consisted of five mice. * indicates statistically significant ($p<0.05$) difference from the control value.

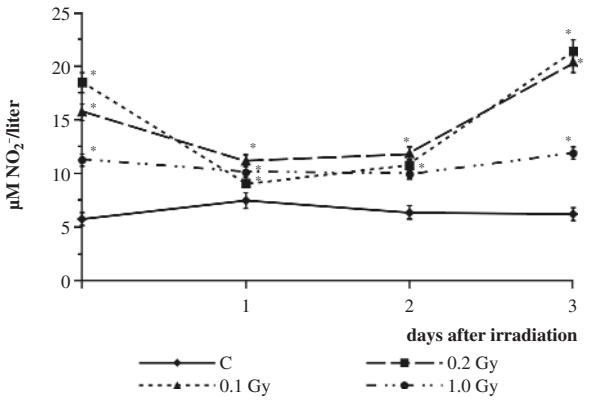

Fig. 4. Production of nitric oxide (NO_2^-) by the IFN-γ- and LPS-treated peritoneal macrophages collected on the day of WBI of mice with 0.1, 0.2 or 1.0 Gy X-rays (2 h post-irradiation) and on the three consecutive days. C – sham-exposed (control) mice; 0.1 Gy – mice exposed to a single WBI with 0.1 Gy X-rays; 0.2 Gy – mice exposed to a single WBI with 0.2 Gy X-rays; 1.0 Gy – mice exposed to a single WBI with 1.0 Gy X-rays. Mean values±SD obtained from three independent experiments are presented; each experimental group consisted of five mice. * indicates statistically significant ($p<0.05$) difference from the control value

post-irradiation of the donor mice with 0.075 Gy X-rays. As suggested by the results reported in our previous publication [24, 25], the stimulatory effect of the exposure to 1.0 Gy X-rays demonstrated in the present investigation may be explained by the possible elimination of radiosensitive T and B cells from the spleen leading to a relative increase in the percentage of the NK effectors in the cytotoxic assay.

In the present study we demonstrate for the first time that exposure of mice to X-rays modulates the expression of FasL on NK lymphocytes collected thereof. Importantly, although all the three doses of X-rays used boosted the NK cell-mediated cytotoxicity, only the low-level irradiations led to the increased expression of the FasL on these cells. The lack of the enhanced expression of FasL after the irradiation with 1.0 Gy X-rays suggests that the mechanism of the cytotoxicity induced by such an exposure is different from that of the NK-mediated cytolysis boosted by 0.1 or 0.2 Gy X-rays.

One of other mediators of cell killing by NK lymphocytes is IFN-γ which, in this process, acts synergistically with tumour necrosis TNF-α. However, we were unable to detect any differences between the cultured NK cells obtained from the irradiated and sham-exposed mice with respect to the IFN-γ synthesis by these cells. The present findings corroborate the results of Kojima et al. [7] who did not detect any significant increase in the production of this cytokine by NK cells collected from mice irradiated with 0.5 Gy X-rays.

In the present study, apart from NK cells, the low-dose radiation-induced increased cytolytic activity was also exhibited by macrophages obtained from the exposed mice. This activity was detectable against both the P815 mastocytoma (a classical target for murine macrophages in the standard cytotoxicity assay) and L1 sarcoma (previously used by us for the induction of the pulmonary tumour colonies) target cells. The effect was accompanied by the elevated production in these cells of NO. Importantly, the stimulated cytotoxicity and synthesis of NO was detectable already on the day of exposure to X-rays and, followed by a decrease on the first and second days, rose again on the third day post irradiation. The low-level radiation-induced stimulation of the production of NO in the IFN-γ- and LPS-treated peritoneal macrophages, accompanied by a significant enhancement of the cytotoxic function of these cells against P815 mastocytoma targets were previously reported by Ibuki and Goto [26, 27] who irradiated mice with 0.04 Gy of gamma rays and examined the selected parameters in cells collected on the day of exposure. In our hands, exposure of mice to 1.0 Gy X-rays did not markedly boost the macrophage-mediated cytotoxicity and production of NO. In contrast, Ibuki and Goto [28] were able to detect the enhanced production of NO in the IFN-γ- or LPS-treated RAW264.7 macrophages irradiated with doses of gamma rays higher than 1.0 Gy but in that study the cells were exposed *in vitro*.

In the present investigation, the stimulated macrophage--mediated cytotoxicity and production of NO after irradiation

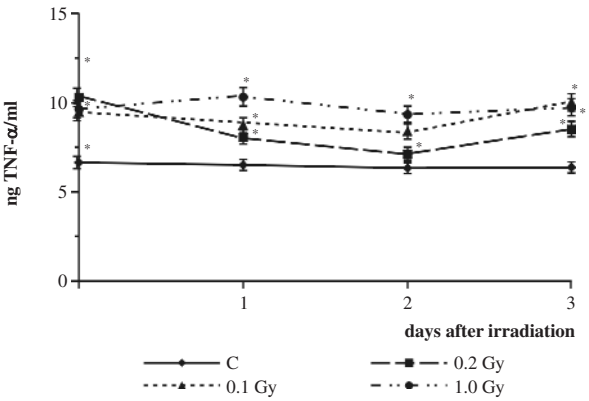

Fig. 5. Synthesis of TNF-α by the IFN-γ- and LPS-treated peritoneal macrophages collected on the day of WBI of mice with 0.1, 0.2 or 1.0 Gy X-rays (2 h post-irradiation) and on the three consecutive days, and then cultured with L1 target cells. C – sham-exposed (control) mice; 0.1 Gy – mice exposed to a single WBI with 0.1 Gy X-rays; 0.2 Gy – mice exposed to a single WBI with 0.2 Gy X-rays; 1.0 Gy – mice exposed to a single WBI with 1.0 Gy X-rays. Mean values obtained from two independent experiments±SD are presented; each experimental group consisted of five mice. * indicates statistically significant ($p<0.05$) difference from the control value

with either of the two low doses of X-rays coincided with the elevated production of TNF-α by these cells. However, unlike in the case of NK cells exposure to 1.0 Gy X-rays did not affect the cytotoxic activity of macrophages but significantly boosted synthesis of TNF-α in these cells. So far, the elevated production of TNF-α by the LPS-stimulated macrophages irradiated with 1.0 Gy γ-rays was described only by Iwamoto & McBride [29] but in this case the cells were exposed *in vitro*.

Overall, the results of the present study indicate that the low-level radiation-induced enhanced cytotoxicity of the two cell types is accompanied by the elevated expression of FasL on the NK lymphocytes and the increased production of NO and TNF-α in the activated macrophages. These observations support and further elaborate the possible mechanism(s) of the involvement of the two cell types in the previously reported by us inhibitory effect of low doses of X-rays on the induction of pulmonary tumour nodules [4, 5]. It remains to be explored in future studies whether other immune cells and/or reactions are also implicated in the tumour-suppressory effects of the low-level exposures to the low-LET radiation.

References

1. Luckey TD (1999): Nurture with ionizing radiation: a provocative hypothesis. Nutr Cancer 34: 1-11.
2. Mitchel RE, Jackson JS, Morrison DP, Carlisle SM (2003): Low doses of radiation increase the latency of spontaneous lymphomas

and spinal osteosarcomas in cancer-prone, radiation-sensitive Trp53 heterozygous mice. Radiat Res 159: 320-327.
3. Shore RE (2001): Radiation-induced skin cancer in humans. Med Pediatr Oncol 36: 549-554.
4. Cheda A, Wrembel-Wargocka J, Lisiak E, et al. (2004): Inhibition of the development of pulmonary tumour nodules and stimulation of the activity of NK cells and macrophages in mice by single low doses of low-let radiation. Int J Low Radiat 1: 171-179.
5. Cheda A, Wrembel-Wargocka J, Lisiak E, et al. (2004): Single low doses of X rays inhibit the development of experimental tumor metastases and trigger the activities of NK cells in mice. Radiat Res 161: 335-340.
6. Barao I, Ascensao JL (1998): Human natural killer cells. Arch Immunol Ther Exp 46: 213-229.
7. Kojima S, Nakayama K, Ishida H (2004): Low dose gamma-rays activate immune functions via induction of glutathione and delay tumor growth. J Radiat Res 45: 33-39.
8. Moretta L, Ciccone E, Poggi A, et al. (1994): Origin and functions of human natural killer cells. Int J Clin Lab Res 24: 181-186.
9. Reyburn H, Mandelboim O, Vales-Gomez M, et al. (1997): Human NK cells: their ligands, receptors and functions. Immunol Rev 155: 119-125.
10. Safwat A, Aggerholm N, Roit I, et al. (2004): Tumour burden and interleukin-2 dose affect the interaction between low-dose total body irradiation and interleukin 2. Eur J Cancer 40: 1412-1417.
11. Smyth MJ, Cretney E, Kelly JM, et al. (2005): Activation of NK cell cytotoxicity. Mol Immunol 42: 501-510.
12. Xu W, Liu LZ, Loizidou M, et al. (2002): The role of nitric oxide in cancer. Cell Res 12: 311-320.
13. Al-Sarireh B, Eremin O (2000): Tumour-associated macrophages (TAMS): disordered function, immune suppression and progressive tumour growth. J R Coll Surg Edinb 45: 1-16.
14. Ibuki Y, Goto R (2004): Ionizing radiation-induced macrophage activation: augmentation of nitric oxide production and its significance. Cell Mol Biol (Noisy-le-grand) 50: OL617-OL626.
15. McKinney LC, Aquilla EM, Coffin D, et al. (1998): Ionising radiation potentates the induction of nitric oxide synthase by IFN-gamma and/or LPS in murine macrophage cell lines: role of TNF-alpha. J Leukoc Biol 64: 459-466.
16. Janik P, Bertram JS, Szaniawska B (1981): Modulation of lung tumour colony formation by a subcutaneously growing tumour. J Natl Cancer Inst 66: 1155-1158.
17. Nagarkatti M, Nagarkatti PS, Kaplan AM (1988): Differential effects of BCNU on T cell, macrophage, natural killer and lymphokine-activated killer cell activities in mice bearing a syngeneic tumor. Cancer Immunol Immunother 27: 38-46.
18. Brunner KT, Engers HD, Cerottini JC: The ^{51}Cr release assay as used for the quantitative measurement of cell-mediated cytolysis in vitro. In: In Vitro Methods in Cell-mediated and Tumor Immunity. Ed. BR Bloom, JR David. Academic Press, London. 1976, 94-106.
19. Shinohara H, Yano S, Bucana CD, Fidler IJ (2000): Induction of chemokine secretion and enhancement of contact-dependent macrophage cytotoxicity by engineered expression of granulocyte-macrophage colony-stimulating factor in human colon cancer cells. J Immunol 164: 2728-2737.
20. Farias-Eisner R, Sherman MP, Aeberhard E, Chaudhuri G (1994): Nitric oxide is an important mediator for tumoricidal activity in vivo. Proc Natl Acad Sci USA 91: 9407-9411.
21. Liu SZ, Su X, Zhang YC, Zhao Y (1994): Signal transduction in lymphocytes after low dose radiation. Chin Med J 107: 431-436.
22. Kojima S, Ishida H, Takahashi M, Yamaoka K (2002): Elevation of glutathione induced by low-dose gamma rays and its involvement in increased natural killer activity. Radiat Res 157: 275-280.
23. Ju GZ, Liu SZ, Li XY, et al.: Effect of high versus low dose radiation on the immune system. In: Radiation Research 1895-1995. Proc. of 10th Intl. Congr. of Radiation Research, Wurzburg, Germany, Aug.27-Sept.1. Ed. U Hagen, D Harder, H Jung, C Streffer. ICRR, Wurzburg, Germany. 1995, 709-714.
24. Harrington NP, Chambers KA, Ross WM, Filion LG (1997): Radiation damage and immune suppression in splenic mononuclear cell populations. Clin Exp Immunol 107: 417-424.
25. Lin IH, Hau DM, Chen WC, Chen KT (1996): Effects of low dose gamma-ray irradiation on peripheral leukocyte counts and spleen of mice. Chin Med J 109: 210-214.
26. Ibuki Y, Goto R (1997): Enhancement of NO production from resident peritoneal macrophages by in vitro gamma-irradiation and its relationship to reactive oxygen intermediates. Free Radic Biol Med 22: 1029-1035.
27. Ibuki Y, Goto R (1995): Augmentation of NO production and cytolytic activity of Mφ obtained from mice irradiated with a low dose of γ-rays. J Radiat Res 36: 209-220.
28. Ibuki Y, Mizuno S, Goto R (2003): γ-irradiation-induced DNA damage enhances NO production via NF-κB activation in RAW264.7 cells. Biochim Biophys Acta 1593: 159-167.
29. Iwamoto KS, McBride WH (1994): Production of 13-hydroxyoctadecadienoic acid and tumor necrosis factor-alpha by murine peritoneal macrophages in response to irradiation. Radiat Res 139: 103-108.