Immune mechanism of the retarded growth of tumor nodules in mice exposed to single low-level irradiations with X-rays

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

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

Abstract

A number of epidemiological and experimental data indicate that exposures to low doses of low-LET ionizing radiation may trigger the activity of natural anti-tumour immune mechanisms and inhibit tumour growth. Natural killer (NK) cells and activated macrophages play an important role in the anti-tumour defence of the host. In our experiments, BALB/c mice were irradiated with single doses of 0.1, 0.2, or 1.0 Gy X-rays and then intravenously (i.v.) injected with L1 sarcoma cells. Cytotoxic activities of NK cells and macrophages were estimated in vitro using the classical ^{51}Cr-release and [^{3}H] thymidine-uptake assays, respectively. The anti-asialo GM1 (GM$_1$Ab) antibody and carrageenan (CGN) were intraperitoneally (i.p.) injected to block the NK cell- and macrophage-mediated activities in vivo, respectively. Whole body irradiation of mice with a single low dose (0.1 or 0.2 Gy) of X-rays led to a significant reduction of the number of tumour colonies induced in the lungs accompanied by the enhanced cytotoxic activities of both NK lymphocytes and macrophages. Treatment of mice with GM1Ab or CGN abrogated the tumour--inhibitory effect of the exposures to 0.1 and 0.2 Gy X-rays. The obtained data suggest that suppression of the development of pulmonary tumour colonies by single irradiations of mice with the two low doses of X-rays may result from stimulation of the natural anti-tumour defence reactions mediated by NK cells and/or cytotoxic macrophages.

Key words: *X rays, low doses, tumour colonies, macrophages, NK cells, cytotoxic activity*

(Centr Eur J Immunol 2006; 31 (1-2): 44-50)

Introduction

As we have been led to believe and according to the doctrine underlying the current radiation protection regulations each exposure to ionizing radiation may lead to the induction of cancer [1]. In fact, solid tumours and leukemias have been detected in people acutely exposed to intermediate to high doses[1] of radiation during nuclear detonations in Hiroshima and Nagasaki or radiotherapy [3-5]. On the other hand, a number of recent epidemiological studies indicate that cancer incidence and mortality are not elevated among inhabitants of the high- versus low-background radiation areas [6, 7]. Moreover, results of the emerging animal studies have demonstrated that whole-body exposures to low doses of X- or γ-rays are associated with the reduced cancer rate and increased latency of spontaneous lymphomas and leukemias in the irradiated subjects [8, 9].

It has been suggested that the anti-neoplastic effects of ionizing radiation may result from stimulation of the tumour surveillance immune mechanisms [10-15] related to the activity of natural killer (NK) lymphocytes, cytotoxic T lymphocytes (CTL), and activated macrophages whose functions are mediated by a variety of cytolytic factors and cytokines [16-27].

In the present investigation we estimated the development of syngeneic pulmonary tumour nodules in mice exposed to single low-level irradiations with X-rays and aimed at the assessment of the possible involvement of NK cells and macrophages in this effect.

Correspondence: Aneta Cheda, Department of Radiobiology and Radiation Protection, Military Institute of Hygiene and Epidemiology in Warsaw, Poland, e-mail: acheda@wp.pl

Material and methods

Animals and irradiation

Male BALB/c mice aged 6-8 weeks were used throughout. The animals, obtained from the Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland, 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 (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 Center and Institute of Oncology, Warsaw, Poland. These cells developed spontaneously in the lungs of a BALB/c mouse and have since been propagated in the *in vitro* culture [28]. 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. 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).

Immunogenicity assay

Mice were intraperitoneally (i.p.) injected with 10^6 L1 sarcoma cells, 10^6 splenocytes obtained from allogeneic C57Bl/6 mice, or pure CM. Four and seven days later the animals were sacrificed and cells from the mesenteric lymph nodes were collected and pulled. After washing, the cells were resuspended in wells of the 96-well culture plate (Corning, Warsaw, Poland) at $3x10^5$ cells per well and incubated for 24 hours at 37°C in a humidified atmosphere of 95% air and 5% CO_2 with 14.8 KBq of [^{3}H]-thymidine (Polatom, Otwock-Świerk, Poland). After that, the cells were washed and their radioactivity was measured in the Tri-Carb 2100TR Counter (Canberra-Packard, Warsaw, Poland). For each experimental group three mice were used.

Lung tumour colony assay

The assay utilizing L1 sarcoma cells was used as a mouse model of experimental tumour metastases. To obtain the cells for the assay, 14 days after s.c. transplantation of 10^6 L1 cells, the developed tumours were removed, minced, and incubated for 30 min. at room temperature with 0.25% trypsin-EDTA (GIBCO BRL) and standard DNase I enzyme solution (Sigma). After that, the cells were washed and resuspended in CM to the final concentration of 10^6 cells/ml. The lung tumour colony assay was performed as described previously [29]. Briefly, two hours after the irradiation mice were i.v. injected with 0.2 ml of the L1-cell suspension per mouse. Fourteen days later, the animals were sacrificed, their lungs injected with India ink and total numbers of superficial macroscopic colonies per lung were counted using a magnifying glass. Each experimental group consisted of 12 mice.

Preparation of the NK cell suspension

NK cells were purified as previously described [30]. 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; in each case the cells were collected and pulled from at least three mice. The non-adherent cells were then collected, washed, and incubated for 30 s at room temperature in the ammonium chloride solution 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.

Preparation of the macrophage-enriched cell suspension

Two days before the collection of cells, mice were i.p. injected with 10% Sephadex G-25 (Pharmacia, Uppsala, Sweden). Peritoneal macrophages were collected on the third day post-irradiation, pulled from at least four mice per each experimental group, resuspended in CM, and incubated on glass Petri dishes for 2 h at 37°C in a humidified atmosphere of 95% air and 5% CO_2. The glass-adherent macrophages were then harvested and resuspended in CM.

NK cell-mediated cytotoxicity assay

Cytotoxic activity of NK cells was measured on the second day post-irradiation using the standard *in vitro* ^{51}Cr-release assay [31]. In brief, 10^6 YAC-1 target cells suspended in 0.1 ml CM were incubated at 37°C in a humidified atmosphere of 95% air and 5% CO_2 for one hour with 5.55 MBq of $Na_2{}^{51}CrO_4$ (Polatom). After the incubation, the cells were washed with PBS and 100-µl aliquots containing 10^4 cells were placed in wells of the microtiter plates (Corning, Warsaw, Poland). The NK-enriched cell populations were then added at the 100:1 effector-to-target (E:T) cell ratio; five samples were performed for each *in vitro* experimental group. After the four-hour incubation at 37°C in humidified atmosphere of 95% air and 5% CO_2, aliquots of the cell-free supernatants were harvested and radioactivity of ^{51}Cr released from the target cells was measured in a γ-counter (Auto-Gamma Cobra II gamma counter; Canberra-Packard). The rate of cytotoxic activity was calculated using

Table 1. Immunogenicity of the allogeneic (AC) and syngeneic (L1) cells

Time after injection of cells	DPM[1]/culture			Index of stimulation[2]	
	CM	AC	L1	AC	L1
day 4	772±54	6021±165	700±42	7.8	0.9
day 7	562±49	5058±139	686±51	9.0	1.2

[1]*Disintegrations per minute (DPM) determined in a beta-counter in the cultures of the mesenteric lymph node lymphocytes obtained from BALB/c mice i.p. injected with pure culture medium (CM), splenocytes from C57Bl/6 mice (allogeneic cells, AC), or L1 sarcoma cells (L1) and incubated in the presence of [3H]-thymidine (as described in Materials and Methods); presented are means ± SD obtained from four replications of each suspension in the experiment; each experimental group consisted of three mice.*

[2]*The ratio of DPM determined in the cultures of mesenteric lymphocytes obtained from mice injected with either AC or L1 cells to DPM of the mesenteric lymphocytes obtained from the CM-injected mice.*

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 release 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 on the third day post-irradiation, as described previously [32], using the L1 sarcoma cells as targets. Briefly, $4x10^6$ L1 cells were suspended in 2.5 ml CM supplemented with 0.3 MBq [^{3}H] thymidine and incubated at 37°C in a humidified atmosphere of 95% air and 5% CO_2 for 20h. The macrophages were then added at the 20:1 effector-to-target (E:T) cell ratio and the CM was supplemented or not with 50 U/ml IFN-γ (Sigma) and 100 ng/ml LPS (Sigma). After the 48-hour 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 taken by target cells when they were cultured alone, and B does those when cultured with effector cells.

Suppression of the NK-cell-mediated activity

To suppress the NK-cell-mediated cytotoxicity *in vivo* the rabbit anti-asialo GM_1 antibody (GM_1Ab; Wako Chemicals, Neuss, Germany) was used as a classical blocker of the activity of murine NK cells [33, 34]. For this purpose, one day before the irradiation and injection of the L1 cells mice were treated i.p. with GM_1Ab (20 μl Ab in 0.5 ml PBS) or 0.5 ml PBS and two or 14 days later assayed for the activity of NK splenocytes and the number of the developed pulmonary tumour colonies, respectively. For each experimental group four (the NK cell-mediated cytotoxicity assay) and 12 (the tumour lung colonies assay) mice were used.

Suppression of the macrophage-mediated activity

To suppress macrophage functions *in vivo* carrageenan (CGN; Sigma) was used as a classical blocker of the activity of these cells [35]. Briefly, one day before the irradiation and four days before the collection of macrophages mice were i.p. injected with CGN (4 mg in 0.4 ml PBS per mice) or 0.4 ml PBS. The animals were then assayed for the number of pulmonary tumour colonies and the collected peritoneal cells were assessed for their cytotoxic activity.

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

Immunogenic characteristics of the syngeneic L1 sarcoma cells and allogeneic C57Bl/6 cells are summarized in Table 1. Based on the study by Ryżewska et al [36], the examined cells can be regarded as immunogenic if the index of stimulation, i.e. the ratio of the activity of [^{3}H]-thymidine incorporated into the mesenteric lymph nodes obtained from mice injected with the cells to the activity of the nodes dissected from mice given only culture medium, exceeds 3.0. Thus, the results shown in Table 1 clearly indicate that L1 sarcoma cells are not immunogenic for the BALB/c mice.

Figure 1 shows rates of the pulmonary tumour colonies (expressed as percentages of the control values obtained in the sham-exposed animals) that grown in mice after the single WBI with various doses of X-rays. As indicated in all the four separate experiments irradiation with 0.1 or 0.2 Gy led to the significant inhibition of the development of the colonies. In contrast, in most of the trials, no statistically significant reduction in the number of pulmonary tumour nodules could be detected when mice were pre-exposed to 1.0 Gy X-rays.

Figure 2 shows the results of the assessments of the *in vitro* cytolytic activity of NK lymphocytes obtained from the spleens of mice two days after exposure to 0.1, 0.2, or 1.0 Gy X-rays compared to the activity of NK splenocytes obtained from the control, sham-irradiated mice. As indicated, irradiation with each of the applied doses of X-rays resulted in the significant boosting of the cytotoxic function of the

NK-type splenocytes. When mice were injected with GM_1Ab the activity of these cells tested two days later was totally abrogated and this inhibition could not be reversed by WBI with 0.1, 0.2 or 1.0 Gy X-rays.

As shown in figure 3, a single WBI of mice with either 0.1 or 0.2 Gy X-rays led to the significant elevation of the cytotoxic activity of the IFN-γ- and LPS-stimulated peritoneal macrophages against the L1 tumour targets on the third day post-exposure to X-rays compared to the activity of these cells obtained from both the sham-irradiated and 1.0 Gy exposed mice. Macrophages collected from mice pre-injected with CGN were significantly less cytotoxic against the L1 cells *in vitro* than macrophages obtained from the CGN-untreated animals in all the examined groups.

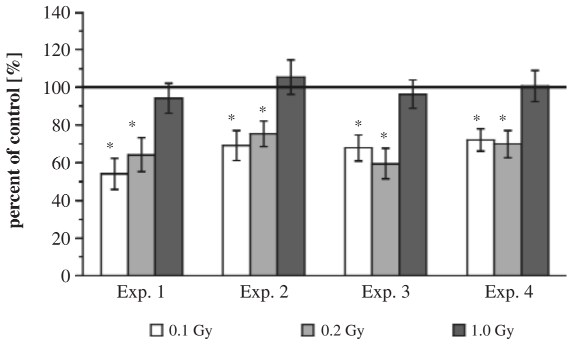

Fig. 1. Relative numbers (percentages of the control values indicated as solid line at 100%) of pulmonary L1 sarcoma cell colonies in mice exposed to 0.1, 0.2 or 1.0 Gy X-rays and two hours later i.v. injected with L1 sarcoma cells; presented are mean values ± SD. Results of four independent experiments (each experimental group consisted of 12 mice) are shown. * indicates statistically significant ($p<0.05$) difference from the control (100%) value

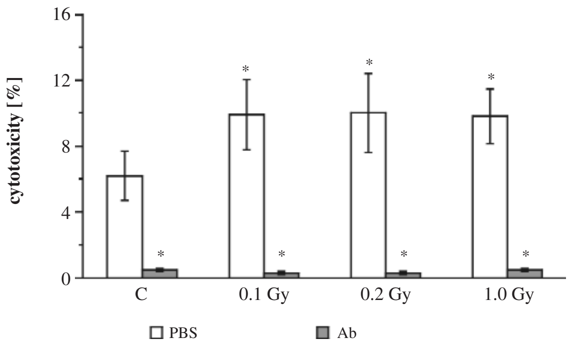

Fig. 2. Cytotoxic activity of splenic NK cells (at 100:1 E:T ratio) on the second day after irradiation of mice with 0.1, 0.2 or 1.0 Gy X-rays. C – sham-exposed (control) mice; 0.1 Gy – mice exposed to a single WBI with 0.1 Gy X-rays; 0.2 Gy – mice exposed to a single WBI with 0.2 Gy X-rays; 1.0 Gy – mice exposed to a single WBI with 1.0 Gy X-rays; PBS – mice i.p. injected with phosphate buffered saline; Ab – mice injected with anti-asialo GM_1 antibody. Presented are means ± SD from three independent experiments; each experimental group consisted of at least three mice. * indicates statistically significant ($p<0.05$) difference from the control value

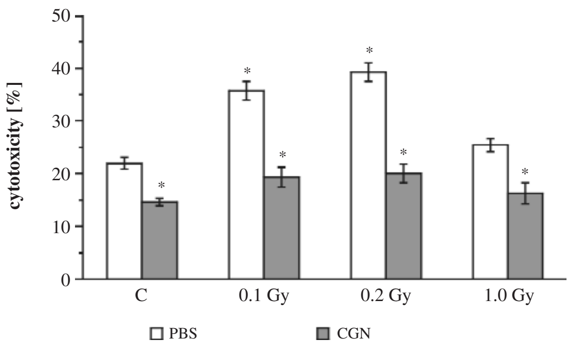

Fig. 3. Cytotoxic activity of peritoneal macrophages (at 20:1 E:T ratio) on the third day after irradiation of mice with 0.1, 0.2 or 1.0 Gy X-rays. C – sham-exposed (control) mice; 0.1 Gy – mice exposed to a single WBI with 0.1 Gy X-rays; 0.2 Gy – mice exposed to a single WBI with 0.2 Gy X-rays; 1.0 Gy – mice exposed to a single WBI with 1.0 Gy X-rays; PBS – mice i.p. injected with phosphate buffered saline; CGN – mice injected with CGN. Presented are means ± SD from three independent experiments; each experimental group consisted of at least three mice. * indicates statistically significant ($p<0.05$) difference from the control value

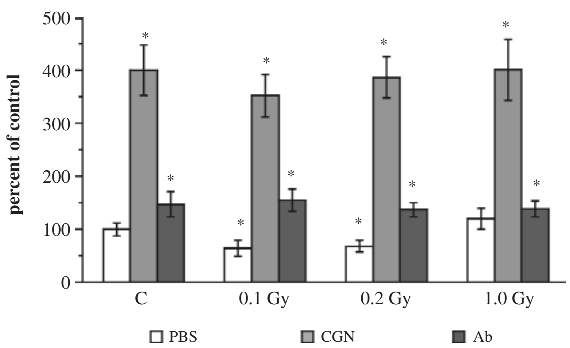

Fig. 4. Relative numbers of pulmonary L1 sarcoma cell colonies in mice exposed to 0.1, 0.2 or 1.0 Gy X-rays and two hours later i.v. injected with L1 sarcoma cells. C – sham-exposed (control) mice; 0.1 Gy – mice exposed to a single WBI with 0.1 Gy X-rays; 0.2 Gy – mice exposed to a single WBI with 0.2 Gy X-rays; 1.0 Gy – mice exposed to a single WBI with 1.0 Gy X-rays; PBS – mice i.p. injected with phosphate buffered saline; CGN – mice injected with CGN; Ab – mice injected with anti-asialo GM_1 antibody. Presented are mean values ± SD. Results of three independent experiments (each experimental group consisted of 12 mice) are shown. * indicates statistically significant ($p<0.05$) difference from the control (C+PBS) value

Figure 4 shows the relative numbers (expressed as percent of the control values measured in the sham-exposed animals) of the pulmonary tumour colonies developed in mice pre-treated with GM_1Ab or CGN. Injection of the NK cell- or macrophage-blocker almost totally eliminated the differences in the numbers of tumour colonies between the irradiated and control groups. This effect was markedly more pronounced in the CGN- than in the GM_1Ab-treated mice.

Discussion

The results of the present study indicate that development of the pulmonary tumour colonies is significantly retarded in mice pre-injected with L1 sarcoma cells and whole body-irradiated with 0.1 or 0.2 Gy of X-rays as compared to the sham-exposed as well as 1.0 Gy-irradiated mice. This observation corroborates the findings of Hosoi and Sakamoto [11] who detected a marked inhibition of both artificial and spontaneous pulmonary metastases in mice inoculated with tumour cells a few hours before or after the exposure to 0.15, 0.2 and 0.5 Gy X-rays. Likewise, significant reduction in the number of pulmonary tumour nodules was reported by Ju et al [12] who irradiated mice with single doses of X-rays ranging from 0.05 to 0.15 Gy 24 hours before the i.v. injection of B16 melanoma or Lewis lung cancer cells. Decreased incidence of lung and lymph node metastases was also reported by Hashimoto et al [14] who exposed rats to 0.2 Gy of γ-rays 14 days after s.c. implantation of hepatoma cells; the same dose, however, did not reduce the number of metastases after local irradiation of the primary tumour nor did it affect the *in vitro* growth of the tumour cells irradiated in the culture medium. Recently, Sakai et al [37] reported that protracted irradiation of mice with γ-rays for over 250 days inhibited the growth of the 20-methylocholantrene-induced tumours. These results collectively suggest that the inhibitory effect of low doses of low-LET radiation on the development of metastases may result from the stimulation of anti-cancer immune mechanisms of the host rather than from the direct reduction of proliferation and/or viability of cancer cells.

NK cells, CTL and activated macrophages are primary effectors of the anti-tumour surveillance system [16, 17, 21, 24, 25, 38]. Since the L1 cells used in the present investigation to induce sarcoma colonies in the lungs are not immunogenic for BALB/c mice specific activity of CTL is not likely to be involved in the described inhibition of the tumour growth by 0.1 and 0.2 Gy X-rays. Hence, it was interesting to note in the present study that the activity of NK cells obtained from mice pre-exposed two days earlier to 0.1 or 0.2 Gy of X-rays was significantly elevated compared the counterpart cells collected from the control, sham-irradiated animals. Similar stimulation was also described by Liu et al [21] 2-6 days after the single exposures of mice to 0.075 Gy and 0.5 Gy X-rays. Interestingly, in our study the activity of splenic NK cells was also markedly stimulated by irradiation with 1.0 Gy X-rays, the dose that did not lead to inhibition of the growth of the pulmonary tumour nodules. However, stimulatory effect of this dose of X-rays on the activity of NK splenocytes could be partially explained by the possible elimination of radiosensitive T and B cells from the spleen and hence the relative increase in the percentage of the NK effectors in the cytotoxic assay (data not shown). In fact, as indicated by Lin et al [39] and Harrington et al [40] NK cells exhibit the highest radioresistance among the splenic lymphoid cells. Moreover, in the present investigation peritoneal macrophages collected on the third day post-irradiation of mice with 0.1 or 0.2 Gy X-rays and incubated in the presence of IFN-γ and LPS exhibited significantly elevated cytotoxic activity against the L1 sarcoma cells. Similar stimulation of the cytotoxicity of the IFNγ- and LPS-treated peritoneal macrophages derived from mice exposed to 0.04 Gy of γ-rays was reported by Ibuki & Goto [18, 19] who used the P815 tumour cells as targets and assayed the effector macrophages already on the day of the exposure.

In the present study i.p. injection of both the anti-asialo GM_1 antibody and CGN suppressed the cytolytic function of NK lymphocytes and macrophages, and abrogated the differences between the numbers of the lung tumour colonies developed in mice exposed to 0.1, 0.2 Gy and 1.0 Gy. These results suggest that stimulation of the NK cell- and macrophage-mediated activities was responsible for the retardation of the development of tumour metastases by the low doses of X-rays. Notably, injection of CGN appeared to be a more potent suppressor of the anti-neoplastic effect of the low-level exposures to X-rays than the GM_1Ab. This observation may be explained by the possible suppression by CGN of the cytotoxic functions of both macrophages and NK cells. Indeed, Minarovits et al [41] demonstrated that concurrent application of the two inhibitors promoted tumor growth in mice transplanted with the SP94 adenocarcinoma and BaF1 fibrosarcoma cells to the same extent as did the sole injection of CGN. Moreover, several cytokines produced by macrophages (e.g., IL-12 and IL-18) are potent modulators of the activity of NK lymphocytes [42] and suppression of the activity of the former may compromise the function of the latter cells.

In conclusion, our present results indicate that suppression of artificial metastases by single low-level irradiations with X-rays may be causatively related to stimulation by such exposures of the cytotoxic functions of NK cells and macrophages. It remains to be explored in future studies whether other immune cells and/or reactions are also involved in the tumour-suppressory effect of the low-dose irradiations with low-LET radiation.

References

1. ICRP Report (2001): A report on progress towards new recommendations: a communication from the International Commission on Radiological Protection. J Radiol Prot 21: 113-123.
2. United Nations: Genetic and Somatic Effects of Ionizing Radiation. United Nations Scientific Committee on the Effects of Atomic Radiation, 1986 Report to the General Assembly, with annexes. United Nations publ. E.86.IX., UN, New York 1986.
3. Pierce DA, Shimizu Y, Preston DL, et al. (1996): Studies of the mortality of atomic bomb survivors. Report 12, Part I. Cancer: 1950-1990. Radiat Res 146: 1-27.
4. Shore RE (2001): Radiation-induced skin cancer in humans. Med Pediat Oncol 36: 549-554.
5. Jagger J (1998): Natural background radiation and cancer death in Rocky Mountain states and Gulf Coast states. Health Phys 75: 428-430.
6. Luckey TD (1999): Nurture with ionising radiation: a provocative hypothesis. Nutr Cancer 34: 1-11.
7. Ishii K, Hosoi Y, Yamada S, et al. (1996): Decreased incidence of thymic lymphoma in AKR mice as a result of chronic, fractionated low-dose total-body X irradiation. Radiat Res 146: 582-585.
8. Mitchel RE, Jackson JS, McCann RA, Boreham DR (1999): The adaptive response modifies latency for radiation-induced myeloid leukemia in CBAH mice. Radiat Res 152: 273-279.
9. 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.
10. Anderson RE, Tokuda S, Williams WL, Warner NL (1982): Radiation-induced augmentation of the response of A/J mice to SaI tumor cells. Am J Pathol 108: 24-37.
11. Hosoi Y, Sakamoto K (1993): Suppressive effect of low dose total body irradiation on lung metastasis: dose dependency and effective period. Radiother Oncol 26: 177-179.
12. Ju GZ, Liu SZ, Li XY, et al.: Effect of high versus low dose radiation on the immune system. In: Radiation Research 1895-1995. The Tenth International Congress of Radiation Research, Wurzburg, Germany, Aug.27-Sept.1, Congress Proc. Ed. U Hagen, D Harder, H Jung, C Streffer. ICRR, Würzburg, Germany. 1995, 709-714.
13. Cai L (1999): Research of the adaptive response induced by low-dose radiation: where have we been and where should we go? Hum Exp Toxicol 18: 419-425.
14. Hashimoto S, Shirato H, Hosokawa M, et al. (1999): The suppression of metastases and the change in host immune response after low-dose total-body irradiation in tumor-bearing rats. Radiat Res 151: 717-724.
15. 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.
16. Barao I, Ascensao JL (1998): Human natural killer cells. Arch Immunol Ther Exp 46: 213-229.
17. 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.
18. 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.
19. 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.
20. Kojima S, Nakayama K, Ishida H (2004): Low dose γ-rays activate immune functions via induction of glutathione and delay tumor growth. J Radiat Res 45: 33-39.
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. Liu XD, Ma SM, Liu SZ (2003): Effects of 0.075 Gy X-ray irradiation on the expression of IL-10 and IL-12 in mice. Phys Med Biol 48: 2041-2049.
23. Mc Kinney LC, Aquilla EM, Coffin D, et al. (1998): Origin and functions of human natural killer cells. J Leukocyte Biol 64: 459-466.
24. 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.
25. Nathan C (1991): Mechanisms and modulation of macrophage activation. Behring Inst Mitt 88: 200-207.
26. Reyburn H, Mandelboim O, Vales-Gomez M, et al. (1997): Human NK cells: their ligands, receptors and functions. Immunol Rev 155: 119-125.
27. Safwat A, Aggerholm N, Roitt 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.
28. 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.
29. Hill RP, Bush RS (1969): A lung colony assay to determined the radiosensitivity of the cells of the solid tumor. Int J Radiat Biol 15: 435-445.
30. 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.
31. 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. Acad. Press. London, 1976, 94-106.
32. Shinohara H, Yano S, Bucana CD, Fidle, 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.
33. Habu S, Fukui H, Shimamura K, et al. (1981): In vivo effects of anti-asialo GM1. I. Reduction of NK activity and enhancement of transplanted tumor growth in nude mice. J Immunol 127: 34-38.
34. Kasai M, Yoneda T, Habu S, et al (1981): In vivo effect of anti-asialo GM1 antibody on natural killer activity. Nature 291: 334-335.
35. Frank J, Born K, Barker JH, Marzi I (2003): In vivo effect of tumor necrosis factor alpha on wound angiogenesis and epithelialization. Eur J Trauma 29: 208-219.
36. Ryżewska AG, Rybicka M, Kania A, Dąbrowski M (1980): Immunogenicity of methylcholantrene-induced tumors tested by draining lymph node assay in syngeneic rats. Neoplasma 27: 533-541.
37. Sakai K, Hosoi Y, Nokamura T, et al. (2003): Suppression of carcinogenic processes in mice by chronic low doses rate gamma-irradiation. Int J Low Radiation 1: 142-146.

38. 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.
39. 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.
40. 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.
41. Minarovits J, Karczag E, Földes I (1989): Enhanced take of spontaneous murine tumors in mice treated with inhibitors of macrophage and/or NK cell function. Neoplasma 36: 3-9.
42. Young HA, Ortaldo J (2006): Cytokines as critical co-stimulatory molecules in modulating the immune response of natural killer cells. Cell Res 16: 20-24.

Footnote

[1]According to the UNSCEAR 1986 Report [2], acute doses above 2 Gy, between 2 and 0.2 Gy, and below 0.2 Gy are regarded as high, intermediate, and low, respectively