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Stimulatory effects of single low-level irradiations with X-rays on functions of murine peritoneal macrophages

Aneta Cheda, Jolanta Wrembel-Wargocka, Ewa M. Nowosielska, Marek K. Janiak

Abstract A number of epidemiological and experimental data indicate that exposures to low doses of low-LET ionising radiation may trigger the activity of natural anti-tumour immune mechanisms and inhibit tumour growth. In the present study, we assessed the cytotoxic activity and production of nitric oxide, superoxide anions, and tumour necrosis factor-α in peritoneal macrophages collected from BALB/c mice exposed to single whole-body irradiations with 0.1, 0.2, or 1.0 Gy X-rays. The results indicate that all the tested parameters were significantly up-regulated in macrophages obtained from mice exposed to 0.1 or 0.2 Gy X-rays but not in those collected from the sham-irradiated and 1.0 Gy-exposed animals.

Key words low doses • X-rays • macrophages • nitric oxide • cytotoxicity • TNF-α

A. Cheda✉, J. Wrembel-Wargocka, E. M. Nowosielska, M. K. Janiak
Military Institute of Hygiene and Epidemiology, Department of Radiobiology and Radiation Protection, 4 Kozielska Str., 01-163 Warsaw, Poland,
Tel.: +48 22-6818518, Fax: +48 22-8104391,
E-mail: acheda@wp.pl

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Introduction

Various epidemiological as well as experimental data from the recent years indicate that exposures to low doses of the low-LET ionising radiation may inhibit tumour growth by triggering the mechanisms of natural immune surveillance [7, 9]. Previously, we showed 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 [2, 3]. This effect might result from stimulation of the activity of cells involved in the anti-tumour surveillance, such as natural killer lymphocytes and activated cytotoxic macrophages, whose functions are mediated by nitric oxide, tumour necrosis factor-α, superoxide anions, and/or other cytolytic factors and cytokines [1, 18].

In view of the important role of activated macrophages in the immune surveillance we aimed in the present investigation to elucidate the effect(s) of irradiation of mice with a low dose of X-rays on the selected parameters of these cells associated with their anti-tumour function.

Material and methods

Animals and irradiations

Male BALB/c mice aged 6–8 weeks were used throughout. The animals were whole-body-irradiated (WBI) using an HS320 Pantak X-ray generator at 2.2 Gy/h dose rate to obtain the absorbed doses of 0.1, 0.2, or 1.0 Gy

per mouse. On the day of the exposure (approx. two hours after the irradiation) and then on the 2nd and 3rd days post-exposure mice were sacrificed. All the experiments were approved by the Local Ethical Committee for Experimentation on Animals at the National Institute of Public Health in Warsaw.

Tumour cells

L1 sarcoma cells were used as targets in the cytotoxicity assays. The cells were maintained in the RPMI-1640 medium supplemented with 10% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine (culture medium, CM).

Harvest of peritoneal macrophages (Mϕ)

Two days before the irradiation, mice were i.p. injected with 1 ml of 10% Sephadex G-25. Peritoneal Mϕ were collected 2 h after the irradiation by vigorous washing of the peritoneal cavity. Following incubation on glass for 2 h at 37°C, 5% CO_2, adherent cells were harvested and resuspended in CM.

Cytotoxicity assay

Cytotoxic activity of Mϕ was measured using the *in vitro* thymidine-release assay as described previously by Shinohara *et al*. [17].

Production of nitric oxide (NO)

Peritoneal Mϕ (2×10^5 cells/well) were incubated for 48 h at 37°C, 5% CO_2 with the addition of 50 U/ml IFN-γ. After that, 100 ml of the supernatants was mixed with the same volume of the Griess reagent and incubated in the dark for 10 min at RT. Absorbance at 540 nm was then measured using a microplate reader.

The nitroblue tetrazolium (NBT) assay

100-μl aliquots of the Mϕ suspension containing 10^7 cells/ml were incubated for 30 min at 37°C, 5% CO_2 with the same volume of the 0.2% solution of NBT. After the incubation and washing, the cells were resuspended in 1 ml of CM supplemented with 20% FBS and microscopic slides were prepared. The slides were fixed with ethanol and stained with 0.2% safranine (Sigma). On each slide, at least 200 cells were scored and macrophages exhibiting clear deposits of diformazan were counted and expressed as fraction of all the analyzed cells.

Synthesis of tumour necrosis factor-α (TNF-α)

Peritoneal Mϕ were incubated for 48 h (37°C, 5% CO_2) with L1 or P815 target cells at the 20:1 ratio. After the incubation, supernatants were collected and assayed for the level of TNF-α using the ELISA test.

Statistical analysis

For statistical analysis of the differences, the Mann-Whitney U test for non-parametric trials was used and p values <0.05 were regarded as significant.

Results and discussion

One of the non-specific, anti-neoplastic immune functions of the organisms is rendered by cytotoxic macrophages [4, 16]. When stimulated by LPS and/or IFN-γ, these cells kill tumour targets via the secreted cytocidal factors, such as NO and TNF-α [4, 6]. In fact, as demonstrated by numerous reports, NO can be primarily involved in the macrophage-mediated cytotoxicity [4, 5] and act as an important inhibitor of the tumour progression [19]. In turn, TNF-α was shown to induce apoptosis in the target neoplastic cells [1, 13].

In the present study, peritoneal macrophages obtained from mice WBI-irradiated with 0.1 or 0.2 Gy X-rays and stimulated with IFN-γ and LPS exhibited the significantly increased cytotoxic activity *in vitro*

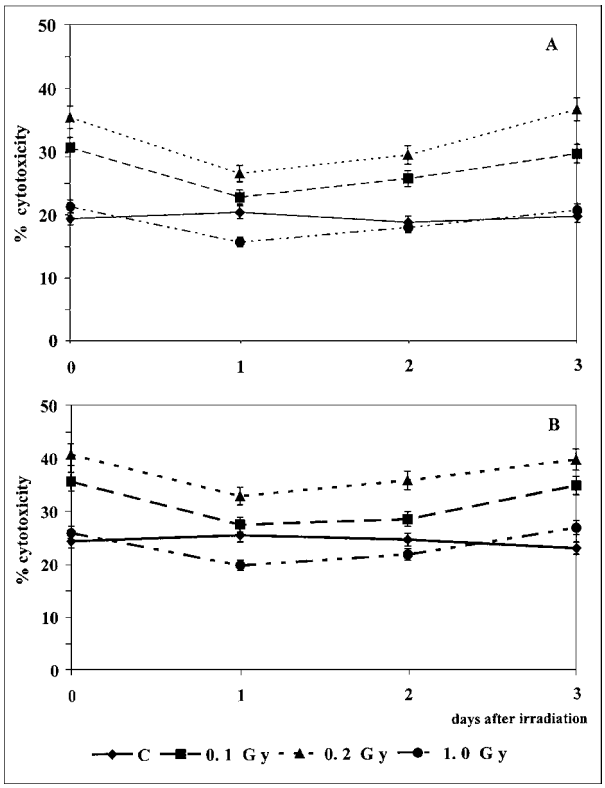

Fig. 1. Cytotoxic activity of naive or IFN-γ-stimulated peritoneal macrophages obtained from mice exposed to various doses of X-rays; A – non-stimulated peritoneal Mϕ, B – Mϕ stimulated with IFN-γ and LPS; C – sham-exposed (control) mice; 0.1 Gy, 0.2 Gy, and 1.0 Gy – mice exposed to a single WBI with 0.1, 0.2, and 1.0 Gy X-rays, respectively. Presented are means ± SD (bars) from three independent experiments; each experimental group consisted of five mice; day '0' refers to data obtained two hours post-irradiation.

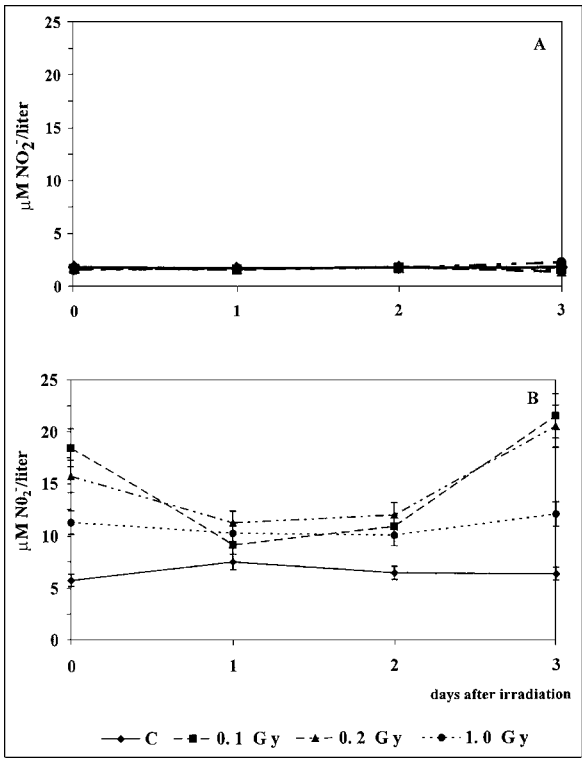

Fig. 2. Production of NO by peritoneal macrophages obtained from mice irradiated with X-rays; A – non-stimulated peritoneal Mϕ, B – Mϕ stimulated with IFN-γ; C – sham-exposed (control) mice; 0.1 Gy, 0.2 Gy, and 1.0 Gy – mice exposed to a single WBI with 0.1, 0.2, and 1.0 Gy X-rays, respectively. Presented are means ± SD (bars) from three independent experiments; each experimental group consisted of three mice; day '0' refers to data obtained two hours post-irradiation.

against the L1 neoplastic targets compared to the cells collected from the sham-irradiated or 1.0 Gy-exposed mice. In the former, two groups of the animals, enhanced cytotoxicity of macrophages (Fig. 1) was accompanied by the significantly elevated production of NO (Fig. 2) and superoxide anions (Fig. 3) in these cells, the effects being detectable as early as 2 hours post-irradiation.

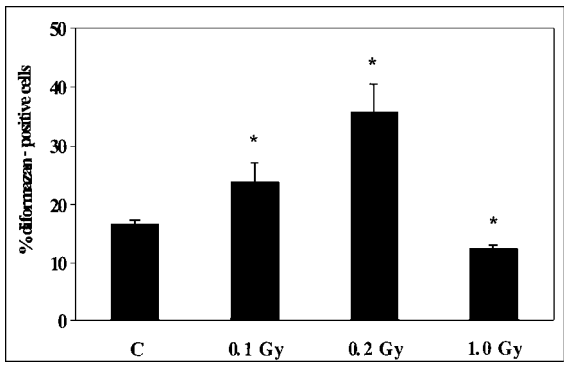

Fig. 3. Reduction of NBT by peritoneal macrophages obtained from mice 2 hours after the irradiation with X-rays; C – sham-exposed (control) mice; 0.1 Gy, 0.2 Gy, and 1.0 Gy – mice exposed to a single WBI with 0.1, 0.2, and 1.0 Gy X-rays, respectively. Mean values ± SD (bars) obtained from two independent experiments are presented; each experimental group consisted of four mice. * statistically significant ($p < 0.05$) difference from the control value.

The low dose-induced stimulation of the NO production by the IFN-γ-primed macrophages accompanied by the significant enhancement of the cytotoxic function of these cells against tumour targets was previously reported by Ibuki and Goto. These authors, however, employed markedly smaller [10, 11] or higher [14] doses of radiation than those applied in the present investigation and used the P815 mastocytoma cells as targets for the cytotoxic macrophages.

In the present investigation, the stimulated cytotoxicity and NO production by macrophages obtained from mice irradiated with either of the two low doses of X-rays coincided with the elevated production of TNF-α by these cells (Fig. 4). In fact, the elevation was detectable already on the day of exposure, followed by a decrease on the first and second days, rising again on the third day post-irradiation. So far, other authors reported that low-level irradiations with gamma rays triggered the macrophage-mediated synthesis of such cytokines as IFN-γ, IL-6, and/or IL-1β [8, 12]. To our knowledge, however, the effects of the low-LET exposures at doses below 0.5 Gy on the macrophage-derived TNF-α have not been described. In fact, in the only study of Iwamoto & McBride [15] demonstrating that macrophages obtained from the irradiated animals and stimulated with LPS produced elevated amounts of

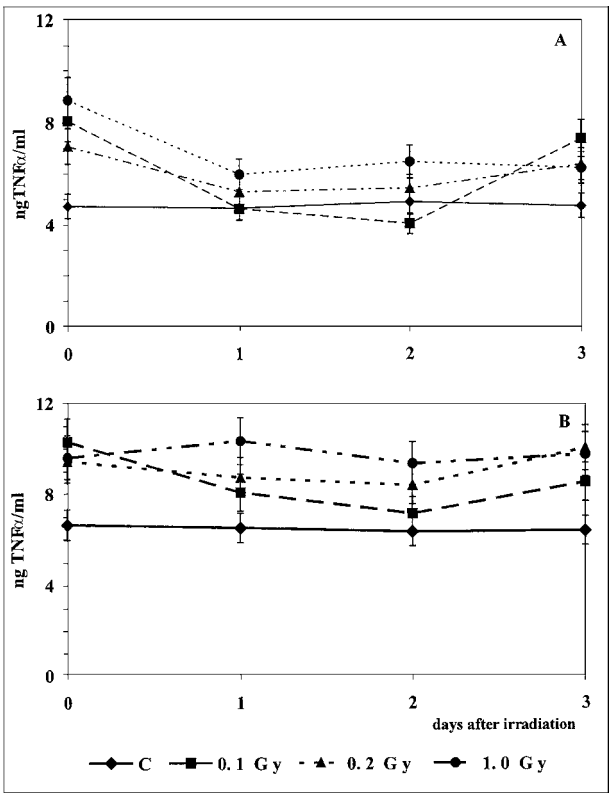

Fig. 4. Synthesis of TNF-α by peritoneal macrophages obtained from mice irradiated with X-ray; A – non-stimulated peritoneal Mϕ, B – Mϕ stimulated with IFN-γ and LPS; C – sham-exposed (control) mice; 0.1 Gy, 0.2 Gy, and 1.0 Gy – mice exposed to a single WBI with 0.1, 0.2, and 1.0 Gy X-rays, respectively. Mean values ± SD (bars) obtained from two independent experiments are presented; each experimental group consisted of five mice; day '0' refers to data obtained two hours post-irradiation.

TNF-α, mice were exposed to doses ranging from 0.5 to 5 Gy of gamma rays. As suggested by the authors, the effect resulted from the radiation-induced changes in the cells' membrane. It remains to be seen in future studies whether similar alterations can account for the enhanced production of TNF-α by macrophages obtained from mice irradiated with X- or gamma rays at doses lower than 0.2 Gy.

Overall, the results of the present study indicate that the previously reported by us inhibitory effect of single low-dose irradiations of mice with X-rays on the induction of pulmonary tumour nodules [2, 3] may be causatively related to stimulation by such exposures of tumoricidal functions of macrophages.

References

1. 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
2. Cheda A, Wrembel-Wargocka J, Lisiak E, Marciniak M, Nowosielska EM, Janiak MK (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
3. Cheda A, Wrembel-Wargocka J, Lisiak E, Nowosielska EM, Marciniak M, Janiak MK (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
4. Cui S, Reichner JS, Mateo RB, Albina JE (1994) Activated murine macrophages induce apoptosis in tumor cells through nitric oxide-dependent or -independent mechanisms. Cancer Res 54:2462–2467
5. 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
6. Frank J, Born K, Barker JH, Marzi I (2003) *In vivo* effect of tumor necrosis factor alpha on wound angiogenesis and epithelization. Eur J Trauma 29:208–219
7. 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
8. Hosoi Y, Miyachi H, Matsumoto Y *et al*. (2001) Induction of inter-leukin-1b and interleukin-6 mRNA by low doses of ionizing radiation in macrophages. Int J Cancer 96:270–276
9. 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
10. 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
11. 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
12. Ibuki Y, Goto R (1999) Contribution of inflammatory cytokine relase to activation of resident peritional macrophages after *in vivo* low-dose γ-irradiation. J Radiat Res 40:253–262
13. 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
14. Ibuki Y, Mizuno S, Goto R (2002) γ-Irradiation-induced DNA damage enhances NO production via NF-kB activation in RAW264.7 cells. Biochim Biophys Acta 1593:159–167
15. 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
16. Nathan C (1991) Mechanisms and modulation of macrophage activation. Behring Inst Mitt 88:200–207
17. 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
18. Wiltrout RH, Herberman RB, Zhang SR *et al*. (1985) Role of organ-associated NK cells in decreased formation of experimental metastases in lung and liver. J Immunol 134:4267–4275
19. Xie K, Fidler IJ (1998) Therapy of cancer metastasis by activation of the inducible nitric oxide synthase. Cancer Metastasis Rev 17:55–75