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

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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

Introduction

Various epidemiological as well as experimental data from the recent years indicate that exposures to low doses of 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% CO₂, 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 × 10⁵ cells/well) were incubated for 48 h at 37°C, 5% CO₂ 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 10⁷ cells/ml were incubated for 30 min at 37°C, 5% CO₂ 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% CO₂) 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 cytoidal 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...
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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. 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.

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