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Low-level exposures to ionising radiation modulate the anti-tumour activity of murine NK cells

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Abstract Experimental evidence from the recent years indicates that low-level irradiations with X- or gamma rays may inhibit development of both primary and secondary tumours and stimulate the activity of natural anti-tumour immune mechanisms. Natural killer (NK) cells play an important role in anti-tumour defence of the host. In the present investigation cytotoxic activity, production of interferon-γ, and expression of the Fas ligand (FasL) were estimated in the NK splenocytes collected from BALB/c mice whose whole body was pre-exposed to irradiation with 0.1, 0.2, or 1.0 Gy X-rays. The results indicate that cytotoxic activity of the irradiated NK cells was significantly stimulated compared to that of the NK effectors obtained from the sham-exposed mice. This effect was totally abrogated by injection of the anti-asialo GM_1 antibody. In addition, compared to the control mice, NK cells obtained from the irradiated animals exhibited reduced surface expression of FasL. Collectively, the obtained results suggest that the inhibitory effect of the low-level irradiations with X-rays on the development of pulmonary tumour nodules may be directly associated with stimulation by such exposures of anti-neoplastic functions mediated by NK cells.

Key words low doses • X-rays • NK cells • cytotoxicity • anti-asialo GM_1 antibody • FasL

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Introduction

The most important late effect of an exposure to ionising radiation is an increased incidence of cancer in the exposed population. On the other hand, experimental evidence from the recent years indicates that low-level irradiations with X- or gamma rays may inhibit the development of both primary and secondary tumours [6, 11, 12, 15]. It has been suggested that this effect is causatively related to the radiation-induced stimulation of such processes as DNA repair, scavenging of free radicals, and/or anti-tumour immune reactions. In fact, results of our previous experiments demonstrated that whole-body irradiation of mice with 0.1 or 0.2 Gy of X-rays led to a significant inhibition of the development of the induced pulmonary tumour colonies [2, 3].

In view of the important role of the naturally cytotoxic lymphocytes (NK cells) in anti-tumour surveillance we sought in the present investigation to determine if single irradiations of mice with low doses of X-rays may affect the activity of these cells in the exposed subjects.

Material and methods

Animals and irradiation

Male BALB/c mice aged 6–8 weeks were used throughout. Single whole-body irradiations (WBI) of the animals were performed with a HS320 Pantak X-ray generator operating at 230 kV, 20 mA, with 1-mm aluminium and 1-mm copper filters, at a dose rate of 2.2 Gy/h so that the absorbed doses were equal to 0.1, 0.2, or 1.0 Gy per mouse. Mice were sacrificed on the 2nd (cytotoxicity assay, determination of FasL and IFN-γ) or 14th day (tumour colonies) post-irradiation. All the animal studies were approved by the Local Ethical Committee for Experimentation on Animals at the National Institute of Public Health in Warsaw.

Antibodies

Twenty μl anti-asialo GM_1 antibody (WAKO Chemicals, Germany) in 0.5 ml PBS (per mouse) was i.p. injected to suppress the NK cell-mediated activity *in vivo*. The FITC-labelled anti-mouse Pan-NK cells antibody (DX5) and the PE-tagged anti-mouse FasL antibody were used to label NK cells in the spleen cell populations obtained from the irradiated and sham-irradiated mice.

Isolation of NK cells

Single-cell suspensions were obtained from the spleens of mice and incubated on glass for 40 min at 37°C, 5% CO_2. Non-adherent cells were then collected, passed through a nylon wool column (to enrich the suspension for NK lymphocytes) and used as effectors in the NK cell-mediated cytotoxicity assay.

Spleen fractions of NK cells

One hundred ml of the NK-enriched cell suspension (10^7 cells/ml) were incubated with 10 μl DX5 antibody for 25 min at room temperature (RT) in the dark and then analyzed in a flow cytometer equipped with the CellQuest software.

The NK cell-mediated cytotoxicity assay

It was performed as described previously [3]. Briefly, YAC-1 target cells were labelled with 5.55×10^6 Bq of ^{51}Cr for 1 h. The cells were then mixed with the NK-enriched cell suspension and incubated at various effector-to-target (E:T) cell ratios for 4 h. Radioactivity released into the supernatant was counted in a γ-counter and the rate of cytotoxic activity was calculated using the formula: % cytotoxicity = [(experimental release – spontaneous release)/(maximum release – spontaneous release)] × 100%.

Suppression of the NK cell-mediated activity *in vivo*

One day before the irradiation and/or the i.v. injection of 2×10^5 L1 tumour cells, mice were treated i.p. with the anti-asialo GM_1 antibody (20 μl Ab in 0.5 ml PBS). These mice were used as sources of the NK-enriched cell suspension for the assessment of the NK cell-mediated cytotoxicity.

Lung tumour colony assay

The lung tumour colony assay was performed as described previously [3]. 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.

Production of interferon-γ (IFN-γ)

The NK-enriched cell suspension was incubated with YAC-1 cells for 4 h. After that, the supernatants were collected and used in the ELISA test (Becton Dickinson, Poland) for estimation of the IFN-γ production by NK cells.

Statistical analysis

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

Results and discussion

In view of the well established role of NK cells in antineoplastic surveillance [1, 13], an important observation of the present study is the significant enhancement of the cytotoxic function of NK-type lymphocytes obtained from mice exposed to a single WBI with 0.1, 0.2 or 1.0 Gy X-rays (Fig. 1). As previously shown by us [2, 3], the highest stimulation was detected 48 h post-irradiation, i.e. approx. at the time when the i.v. injected tumour cells extravasate from the pulmonary capillaries and migrate into the stroma [14]. Triggering of the activity of NK cells after the WBI with 0.075 to 0.5 Gy X- or γ-rays was previously reported by Liu *et al*. [10], Ju *et al*. [5], and Kojima *et al*. [7]. However, the present investigation is the first to associate the previously demonstrated by us suppressed development of experimental pulmonary tumours after a single low-level exposure to X-rays [2] with the markedly stimulated function of NK cells. In fact, as demonstrated in the present study, injection of the anti-asialo GM_1 antibody almost totally inhibited the NK cell-mediated activity *in vivo* and abrogated the differences between the numbers of tumour colonies in the lungs of the control mice and those exposed to 0.1, 0.2, or 1.0 Gy of X-rays (Fig. 2).

A single WBI of mice with 1.0 X-rays led to changes in the fraction of NK cells in the spleen; this effect was

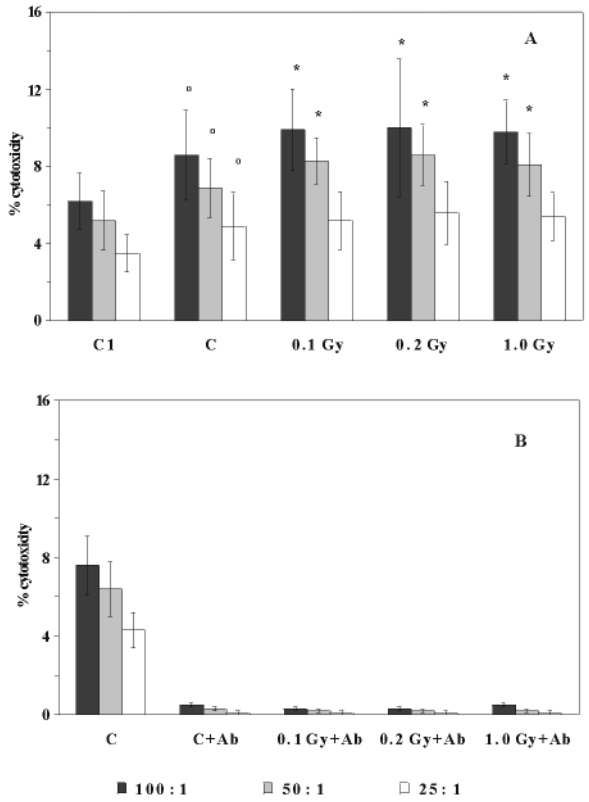

Fig. 1. NK cell-mediated cytotoxic activity: two days after a single WBI of mice with X-rays (A), two days after a single WBI of mice with X-rays and i.p. injection of the anti-asialo GM_1 antibody (B). Ab – injection of the anti-asialo GM_1 antibody; C1 – sham-exposed (control) mice; C – sham-exposed mice injected with L1 sarcoma cells; 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, and injected with L1 cells. 100:1, 50:1, 25:1 – effector to target cell ratio. Presented are means ± SD (bars) from three independent experiments; each experimental group consisted of three mice. ° statistically significant ($p < 0.05$) difference from the results obtained in C1; * statistically significant ($p < 0.05$) difference from the results obtained in C.

not detected after the exposure to 0.1 or 0.2 Gy X-rays (data not shown). Since NK splenocytes are relatively insensitive to ionising radiation [4, 9], the increase in the percentage of these cells among the tested splenocyte population could result from the 1.0 Gy-induced depletion of the more radiosensitive spleen cells, such as B and T lymphocytes. However, in view of the report of Takeuchi and Shibata [16] who found that doses around 1.0 Gy and higher of soft X-rays significantly reduced the number of the asialo-GM_1-positive cells in the spleens of mice, this suggestion needs verification in future studies.

As the cytolytic activity of NK cells may be triggered by FasL [1, 13], we sought to establish if the expression of this ligand on the effector splenocytes can be modulated by a low-level WBI of mice with X-rays. We found that NK lymphocytes obtained from mice exposed to 0.1 and 0.2 Gy demonstrated increased expression of the surface FasL as compared to the sham-irradiated and 1.0 Gy-exposed counterparts (Fig. 3). To our knowledge, this is the first observation of this kind.

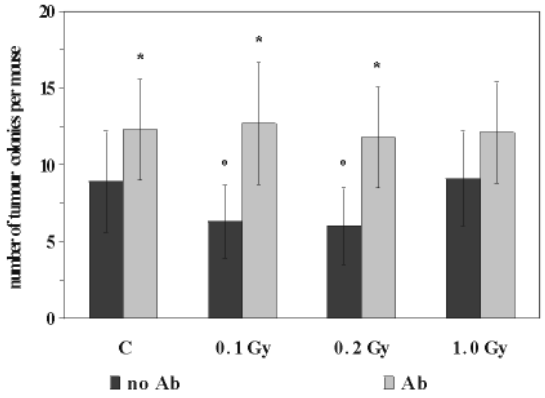

Fig. 2. Numbers of the tumour colonies in the lungs of mice pretreated or not with the anti-asialo GM_1 antibody (Ab) and injected with L1 sarcoma cells; C – sham-exposed (control) mice; 0.1 Gy – mice exposed to a single WBI with 0.1 Gy X-rays; 0.2 Gy – mice exposed to a single WBI with 0.2 Gy X-rays. Mean values obtained from two independent experiments ± SD (bars) are presented; each experimental group consisted of 12 mice. * statistically significant ($p < 0.05$) difference from the non-Ab-treated mice; ° statistically significant ($p < 0.05$) difference from the results obtained in the control, non-Ab injected mice.

One of the mediators of cell killing by NK lymphocytes is IFN-γ which, in this process, acts synergistically with tumour necrosis factor-α. However, we were unable to detect any differences in the production of IFN-γ by the cultured NK cells obtained from the irradiated and sham-exposed mice. This observation corroborates the findings of Kojima *et al.* [8] who did not detect any significant increase in the production of this cytokine by T and NK cells after the WBI of mice with 0.5 Gy X-rays.

Overall, the obtained results indicate that the inhibitory effect of the single low-level exposures to X-rays on the development of pulmonary tumour nodules

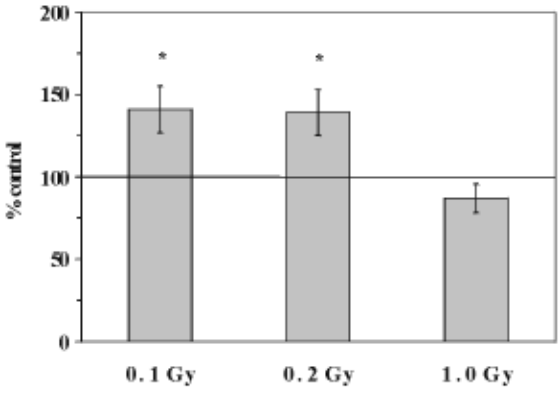

Fig. 3. Relative (percentage of the control value) surface expression of FasL on NK cells two days after a single WBI of mice with X-rays. 0.1 Gy, 0.2 Gy, and 1.0 Gy – mice exposed to a single WBI with 0.1, 0.2, and 1.0 Gy X-rays, respectively, and injected with L1 cells. Presented are means ± SD (bars) from three independent experiments; each experimental group consisted of three mice. * statistically significant ($p < 0.05$) difference from the control (100%) value (sham-exposed mice injected with L1 sarcoma cells).

[1, 14] may be causatively related to stimulation by such exposures of the anti-neoplastic activity of NK cells.

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