Effect of Low Doses of Low-LET Radiation on the Innate Anti-tumor Reactions in Radioresistant and Radiosensitive Mice

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Epidemiological data:

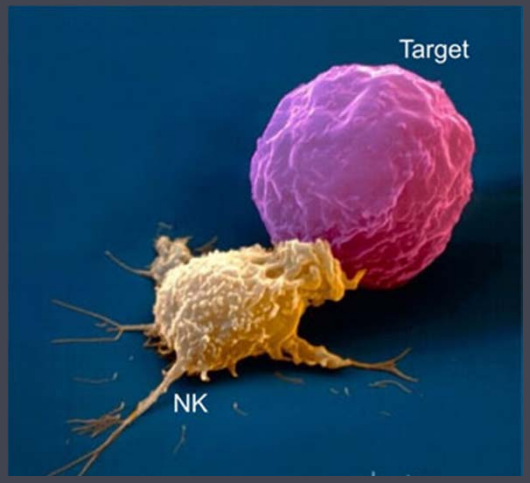
- no evidence of increased cancer morbidity in elevated background radiation regions (China, India, Iran);
- lower incidence of leukemia and some solid tumors among nuclear industry and radiology workers (USA, Canada, Great Britain) exposed to low doses of ionizing radiation, as well as in Hiroshima and Nagasaki nuclear bomb survivors irradiated with ≤ 0.25 Gy;
- decreased lung cancer morbidity among fluoroscopic patients;
- decreased lung cancer morbidity among tenants of homes with elevated radon.

Experimental data:

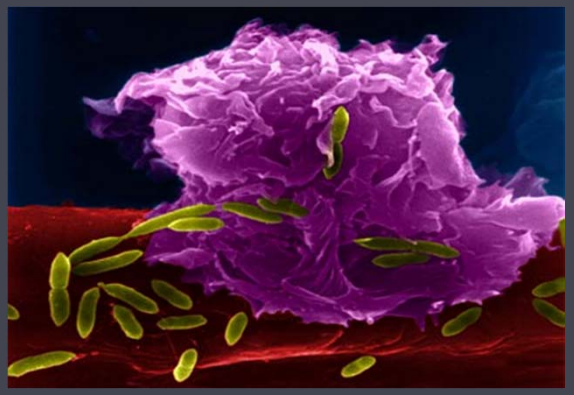
- prolonged life of lab animals;
- decreased tumor progression in laboratory animals (even in those exposed before inoculation of tumor cells);
- inhibition of growth of pulmonary tumor nodules after i.v. injection of neoplastic cells;
- inhibition of spontaneous tumor metastases in lungs or lymph nodes after s.c. injection of neoplastic cells.

Cellular effectors of innate, non-specific anti-tumor surveillance

- NK lymphocytes:
  - perforins
  - granzymes
  - IFN-γ
  - FasL



- Cytotoxic macrophages:
  - NO
  - IL-1β
  - TNF-α
  - IL-12



BALB/c mice

- relatively radiosensitive, cancer-prone *(Storer et al. 1988)*
- elevated incidence of radiation-induced genomic instability *(Ponnaiya et al. 1997)*
- Inherently sensitive to transforming effect of ionizing radiation *(Ullrich and Ponnaiya 1998)*
- inefficient non-homologous end joining of γ-ray-induced DSBs *(Okayasu et al. 2000)*
- Th2/M2 phenotype (anti-inflammatory) *(Mills et al. 2000)*

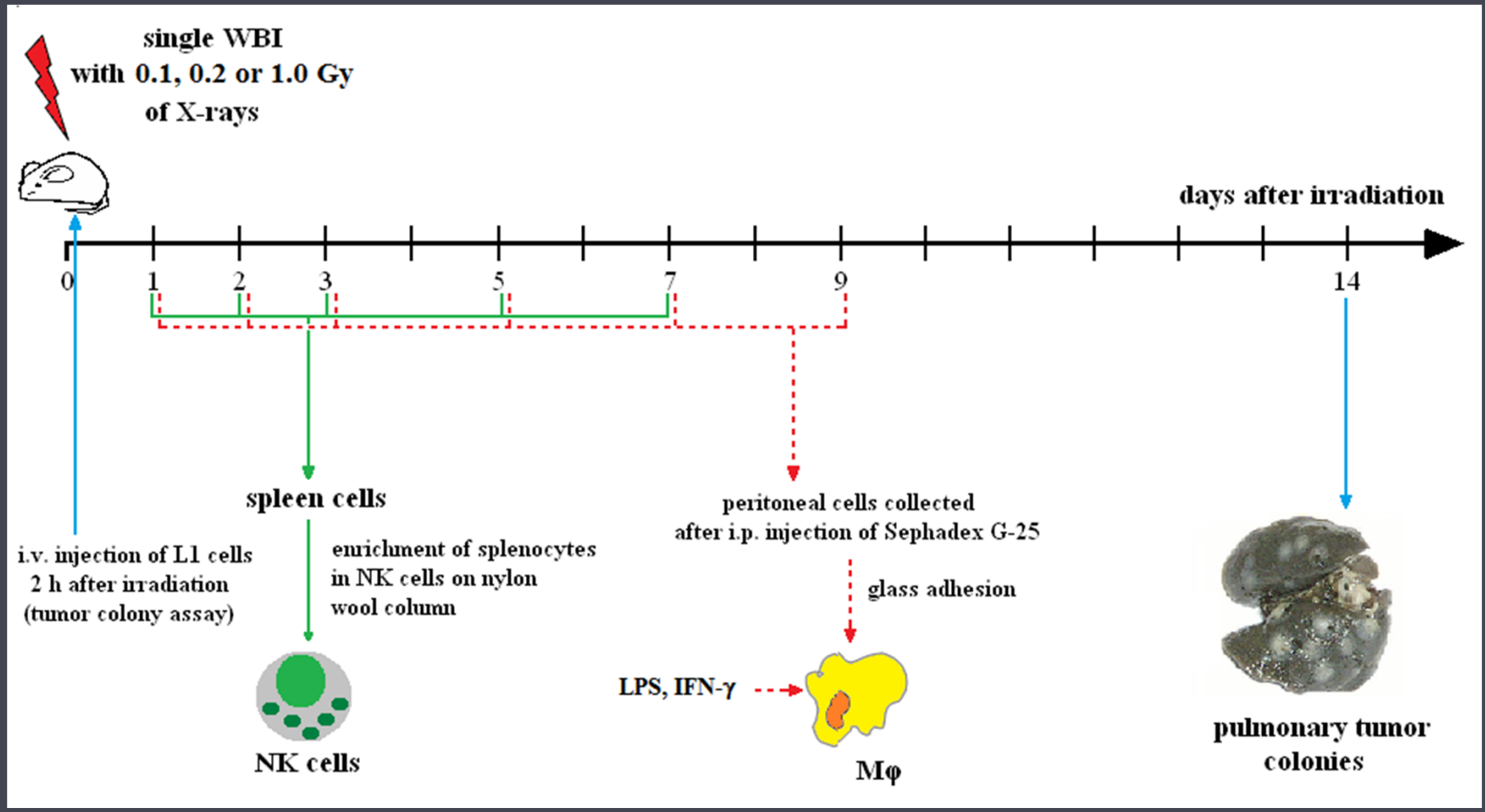
C57BL/6 mice

- relatively radioresistant *(Storer et al. 1988)*
- low incidence of radiation-induced genomic instability *(Ponnaiya et al. 1997)*
- inherently less sensitive to the transforming effect of ionizing radiation *(Ullrich and Ponnaiya 1998)*
- efficient non-homologous end joining of γ-ray-induced DSBs *(Okayasu et al. 2000)*
- Th1/M1 phenotype (pro-inflammatory) *(Mills et al. 2000)*

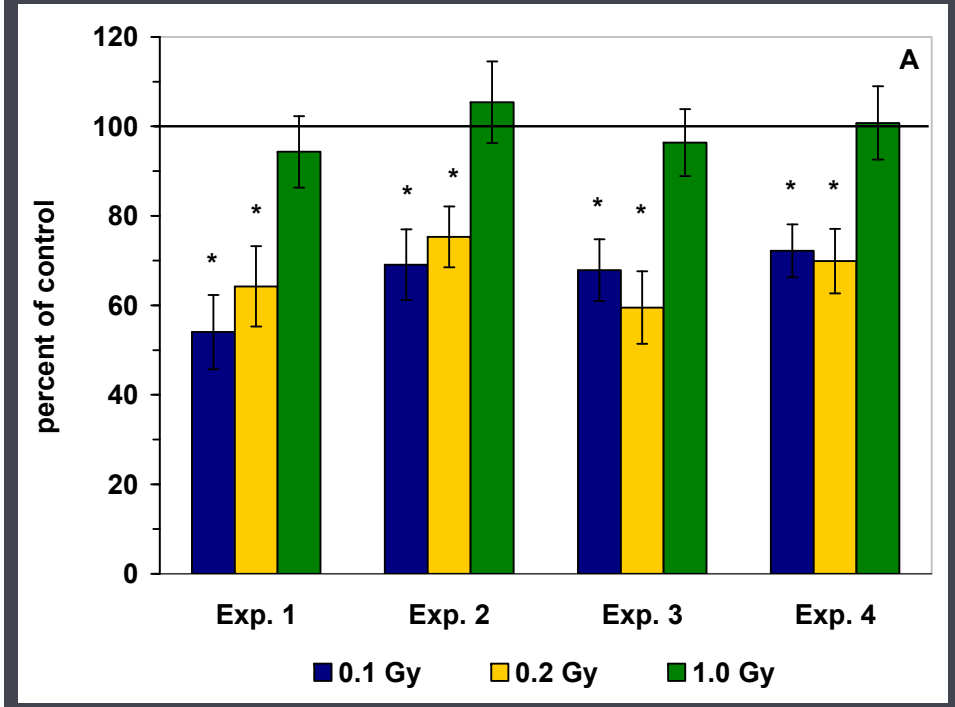
Objectives

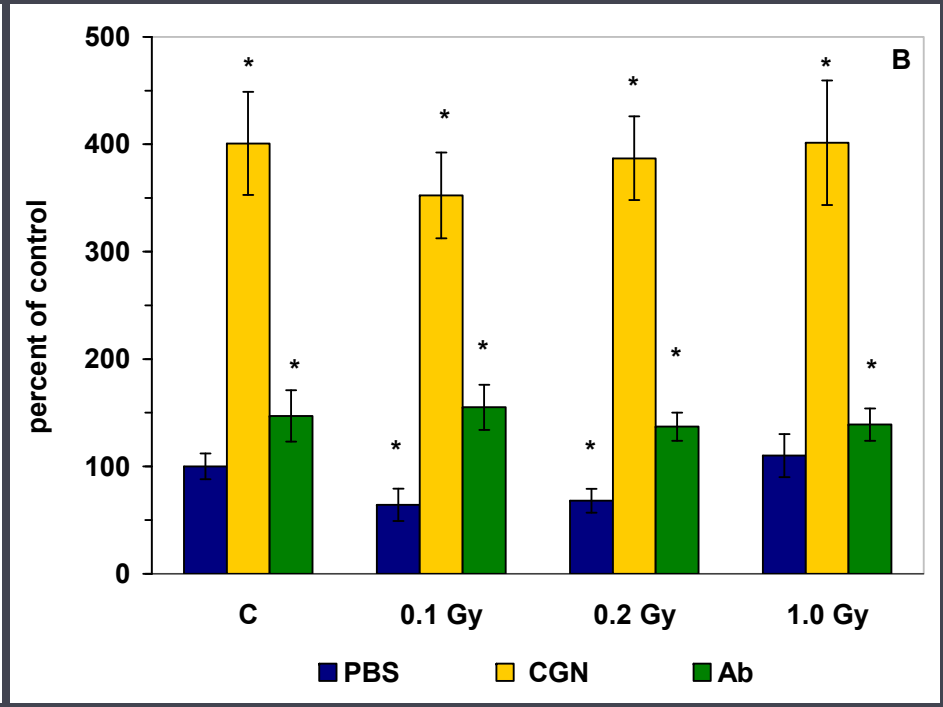
Assessment of the effects of low-level X-ray exposures of BALB/c and C57BL/6 mice on the development of induced neoplastic colonies and activities of innate anti-tumor cells

Experimental protocol: single whole-body irradiation



Development of induced metastases in the lungs after single WBI of BALB/c mice (A) and inhibition of NK cells or Mφ (B)





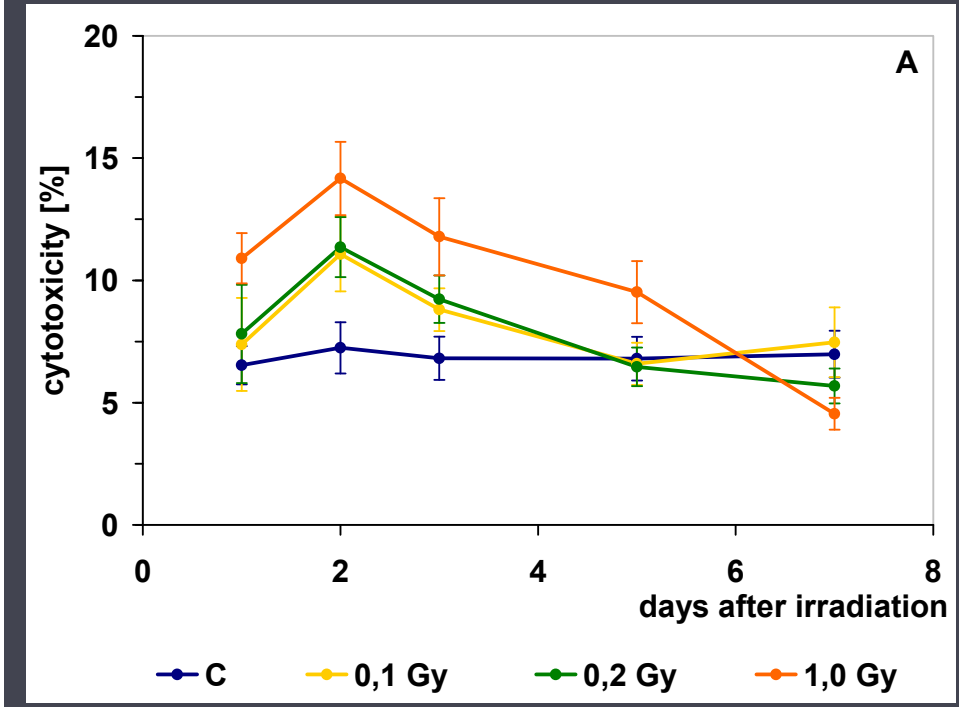
Single WBI of BALB/c mice with 0.1 or 0.2 Gy of X-rays inhibits development of pulmonary metastases

Anti-asialo GM_1Ab and CGN eliminate differences in numbers of lung tumor colonies between irradiated and control groups.

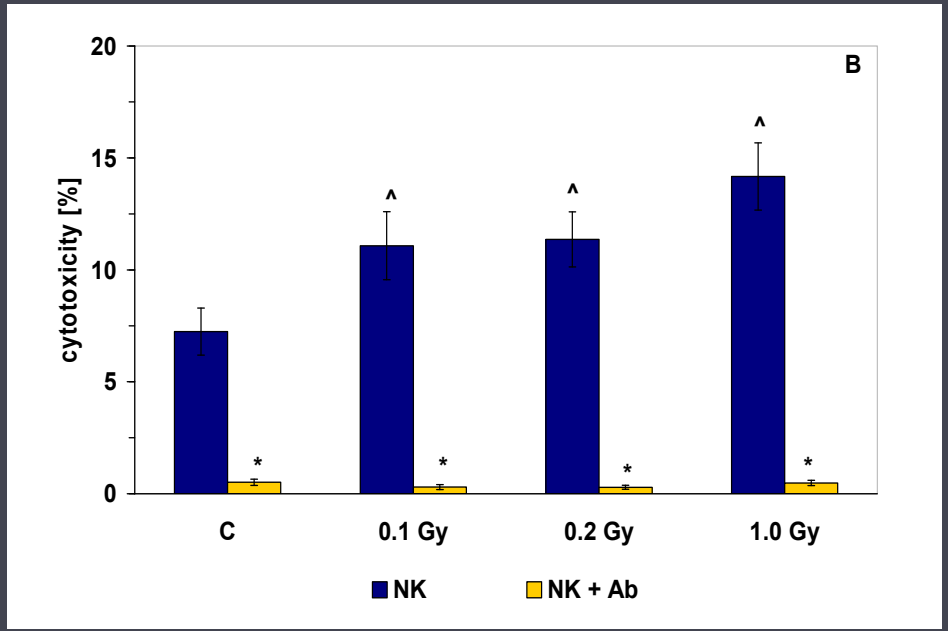
Spontaneous and induced metastases in the lungs of other mouse strains after single WBI

- Marked reductions in the numbers of both artificial and spontaneous pulmonary metastases after single WBI of WHT/Ht mice with 0.15, 0.2, or 0.5 Gy X-rays; inhibitory effect expressed when tumor cells inoculated a few hours before or after the exposure *(Hosoi and Sakamoto 1993)*;
- Significant retardation of development of pulmonary tumor nodules in C57BL/6 mice irradiated with single low doses of X-rays (0.05-0.15 Gy) 24 h before i.v. injection of B16 melanoma or LLC cells *(Ju et al. 1995, Cai 1999)*.

Cytotoxic activity of NK cells (A) and its suppression (B) after single WBI of BALB/c

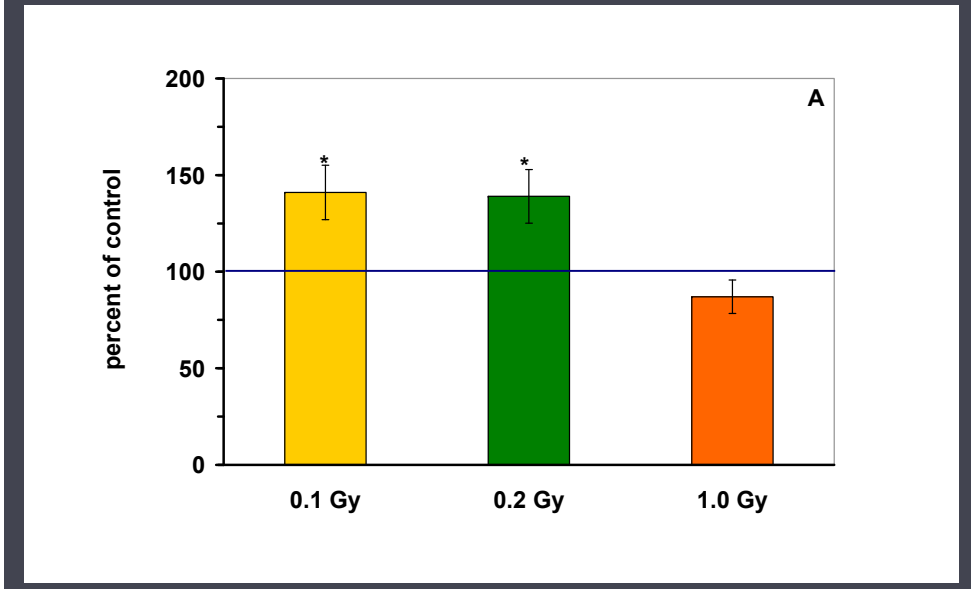


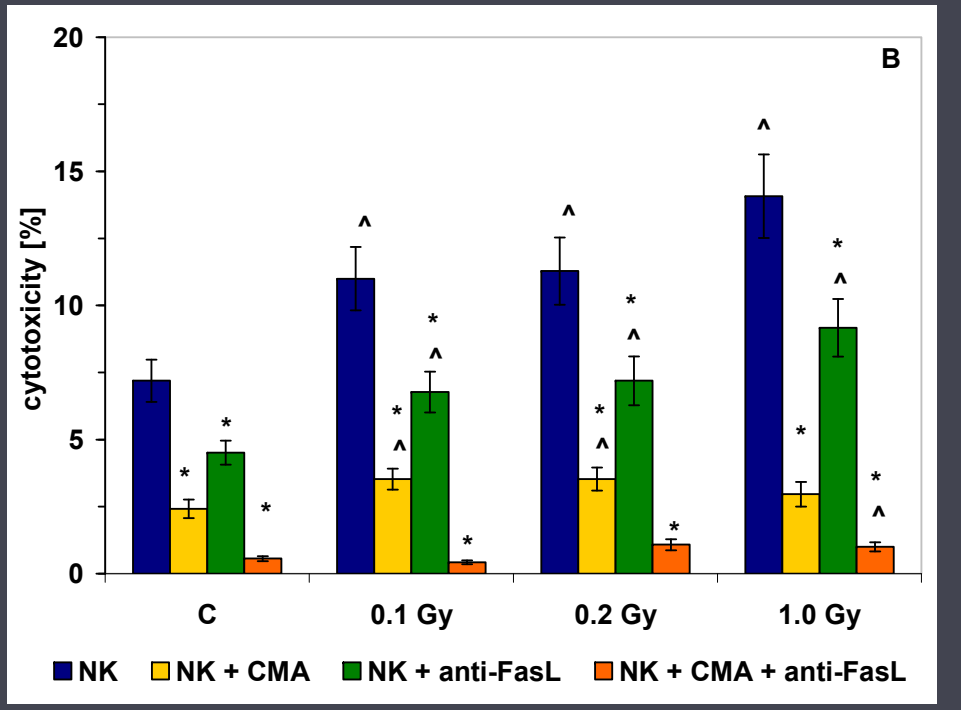
Significant stimulation of cytotoxic activity of NK cells after single WBI of BALB/c mice at 0.1, 0.2, or 1.0 Gy



Injection of anti-asialo GM_1Ab totally abrogates cytotoxic activity of NK cells; the effect not reversed by WBI with 0.1, 0.2 or 1.0 Gy

Surface expression of FasL (A) and Inhibition of cytotoxic activity of NK cells (B) after single WBI of BALB/c mice

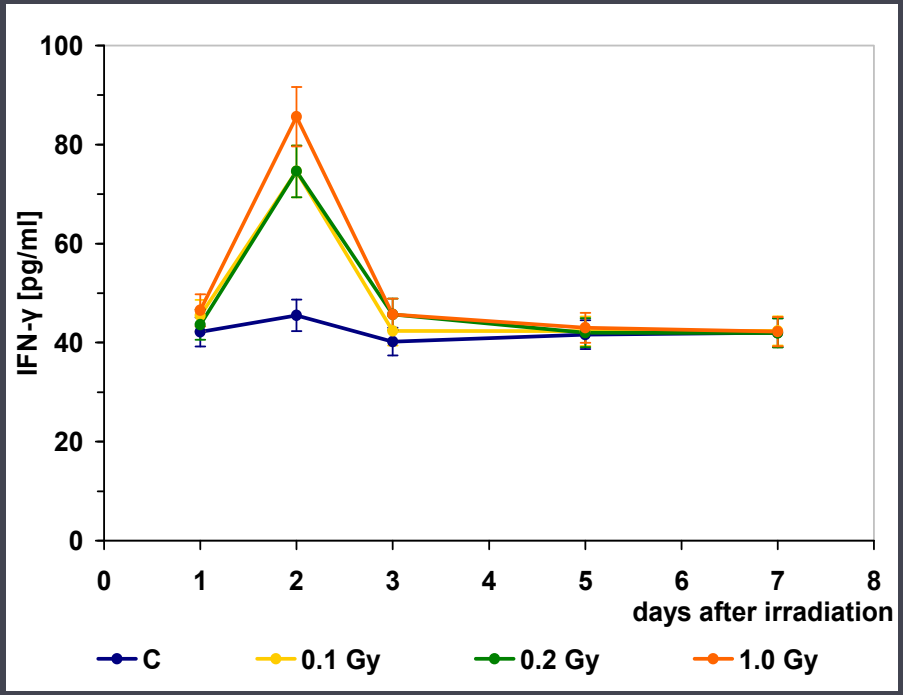


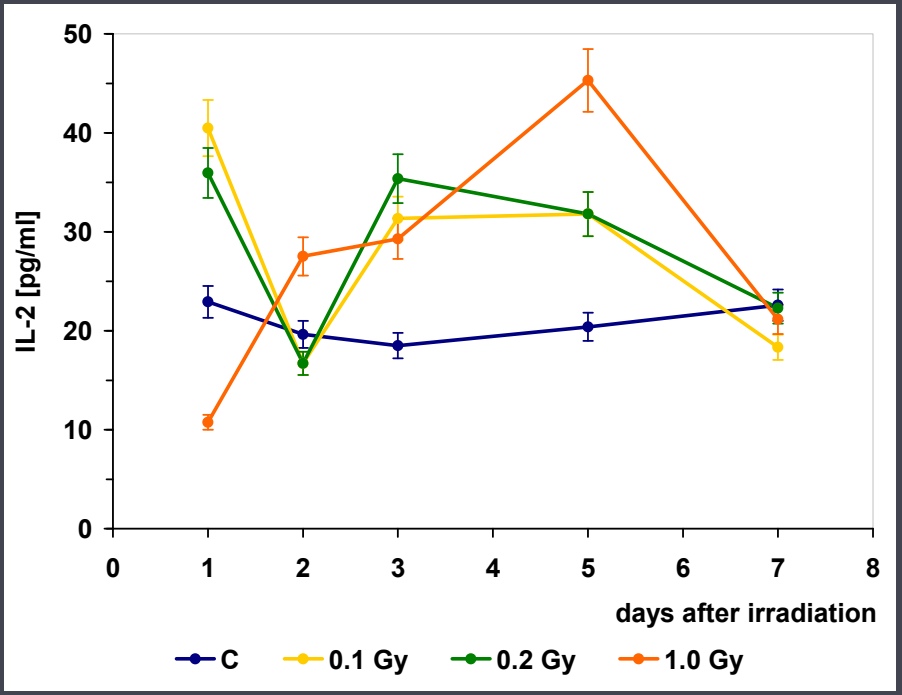


Elevated cytotoxicity of NK cells after single WBI at 0.1, 0.2, or 1.0 Gy is mediated by perforin and FasL.

Exposure to 0.1 and 0.2 Gy X-rays, but not to 1.0 Gy, increases surface expression of FasL on NK cell-enriched splenocytes.

Production of IFN-γ by NK cell-enriched splenocytes and IL-2 by splenocytes after single WBI of BALB/c mice



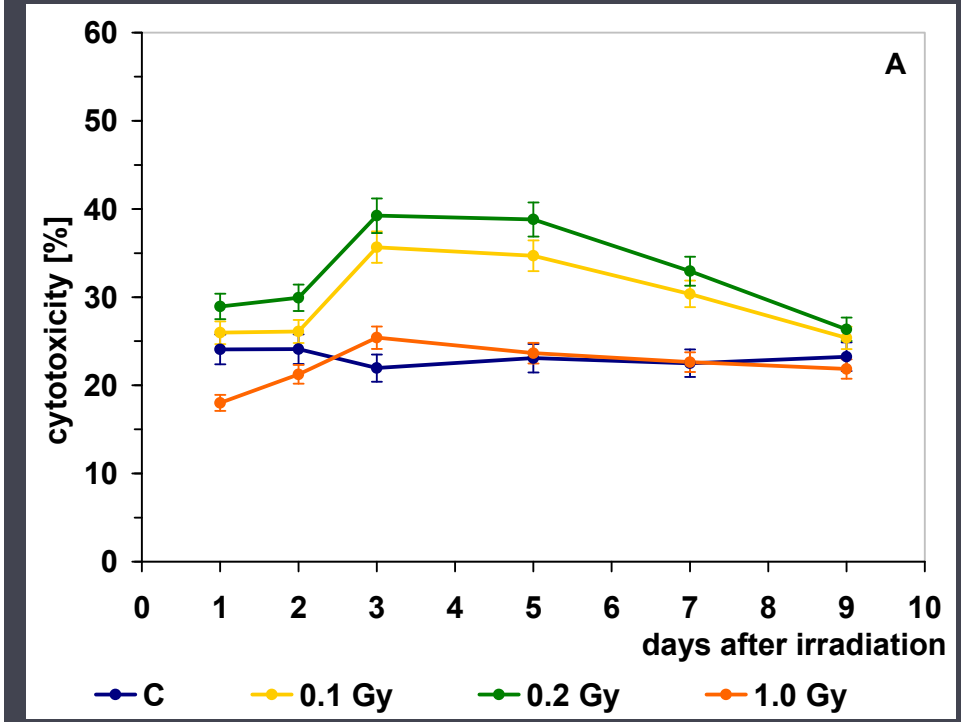


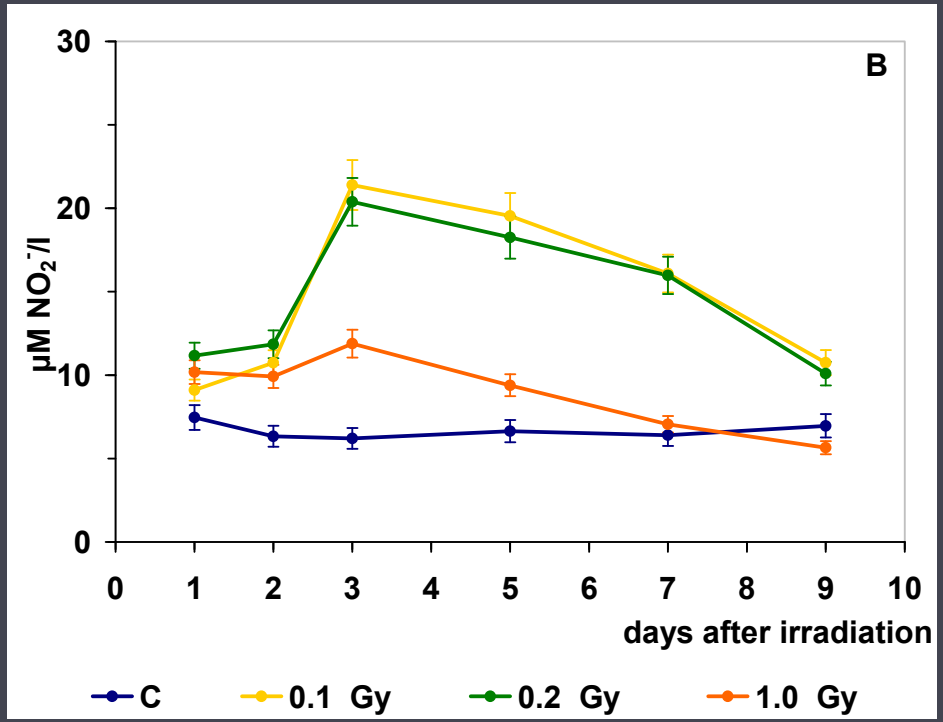
Single WBI of mice at 0.1, 0.2, or 1.0 Gy X-rays stimulates production of IFN-γ by NK cell-enriched splenocytes and IL-2 by splenocytes.

Anti-tumor activity of NK cells after single WBI of other mouse strains

- Stimulation of NK cell-mediated cytotoxicity 24 h after single exposure of C57BL/6 mice at 0.075 Gy X-rays *(Liu et al. 1994)*.
- Boosting of NK cell-mediated cytotoxicity 4 to 6 h post-exposure of ICR mice to 0.5 Gy γ-rays *(Kojima et al. 2002, 2004)*.
- Enhancement of cytocidal function of murine NK-type lymphocytes 2-6 d after single WBI of C57BL/6 mice at 0.075 Gy X-rays *(Ju et al. 1995)*.
- Elevated production of IL-2 and IFN-γ after single irradiation of C57BL/6 mice with 0.075 Gy X-rays *(Fu et al. 1996,1997, Zhang et al. 1999, Liu et al. 1994)* or 0.04 Gy γ-rays *(Ibuki and Goto 1995)*

Cytotoxic activity of Mφ (A) and production of NO by Mφ (B) after single WBI of BALB/c mice

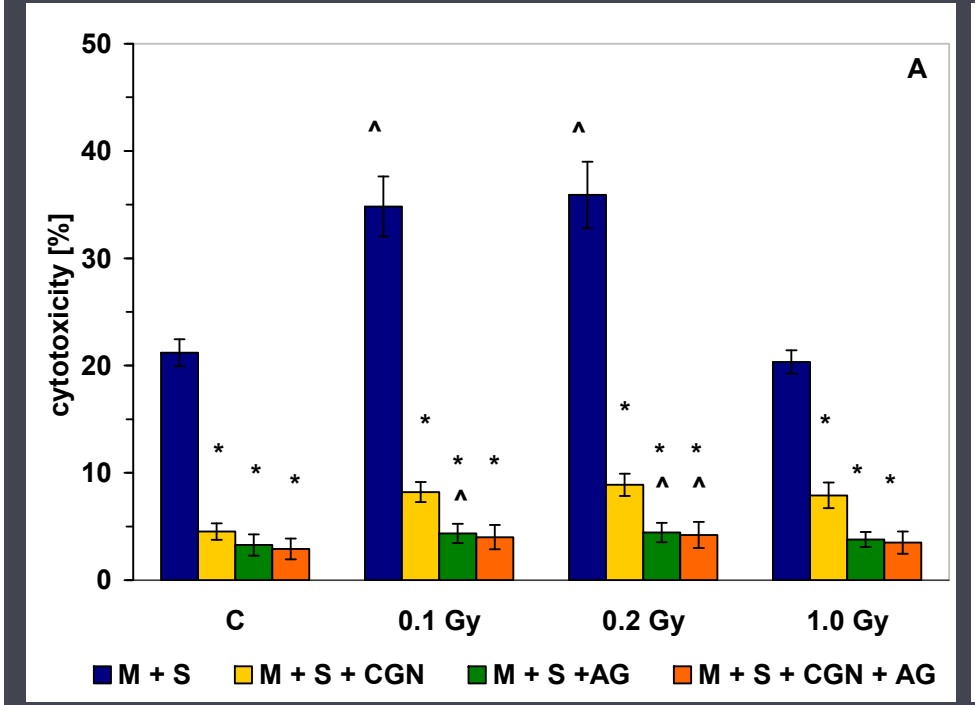


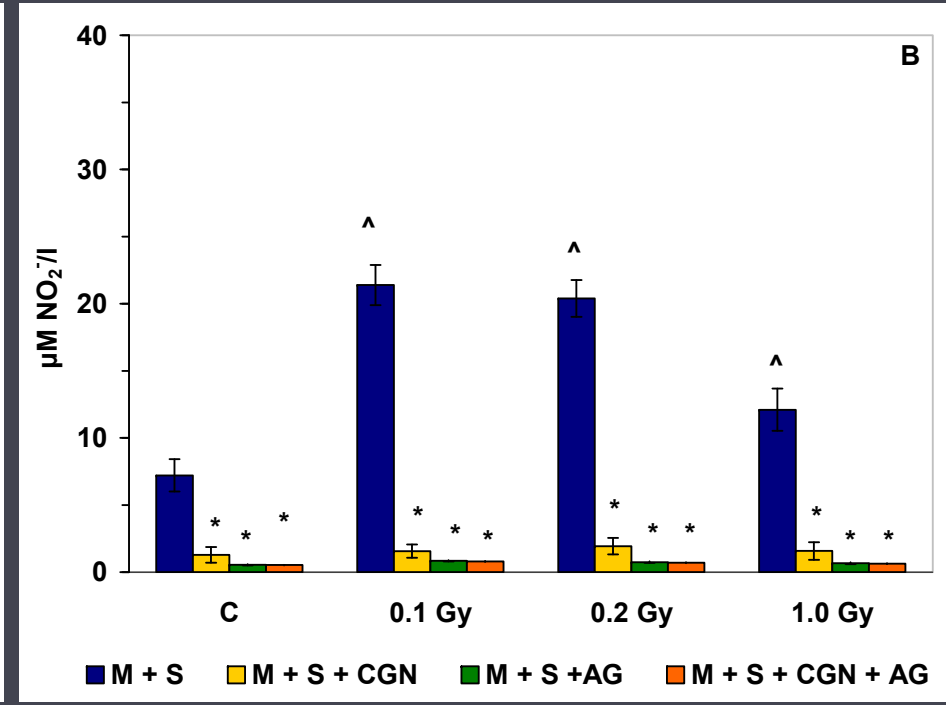


Single WBI at 0.1 or 0.2 Gy X-rays significantly stimulates Mφ-mediated cytolysis of L1 cells.

Single WBI at 0.1 or 0.2 Gy X-rays significantly stimulates production of NO by peritoneal Mφ.

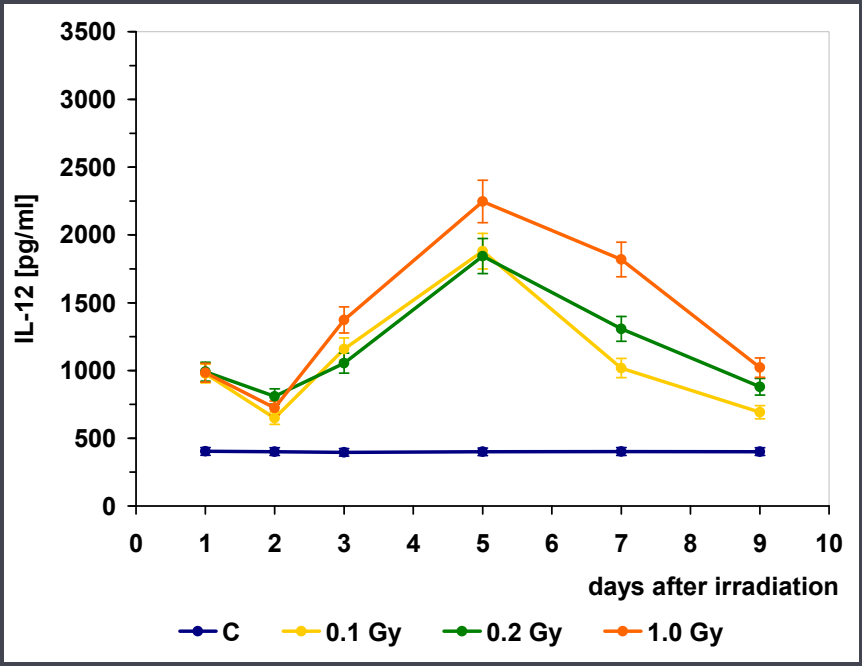
Inhibition of cytotoxic activity (A) and production of NO (B) by Mφ after single WBI of BALB/c mice

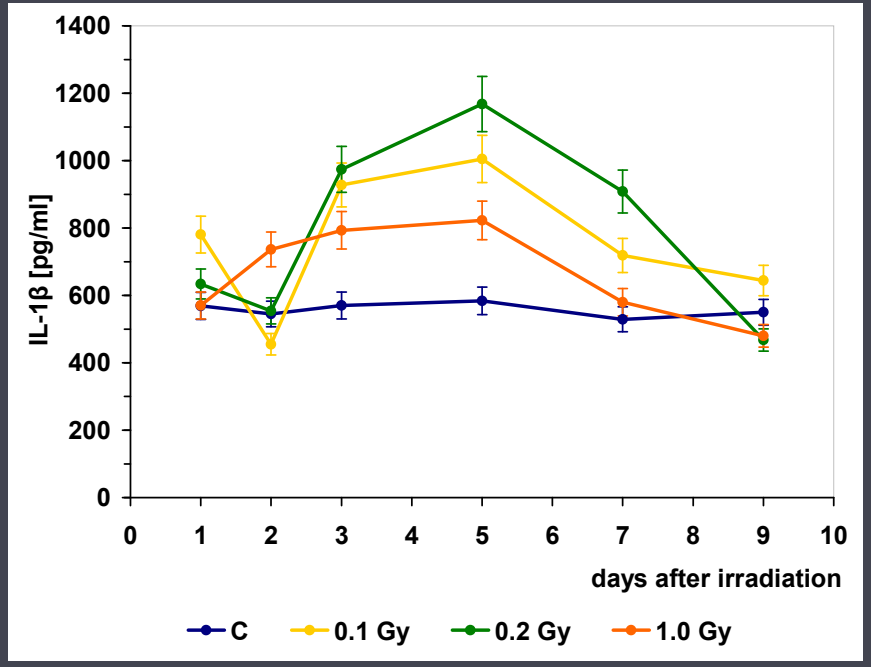


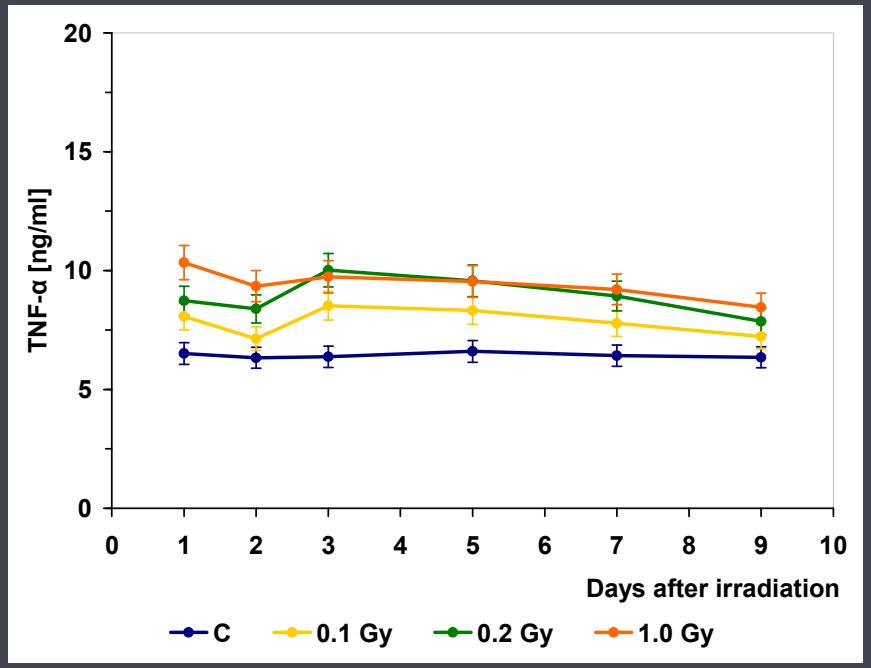


The *in vivo* suppression of Mφ by CGN significantly inhibits the Mφ-mediated cytotoxicity and NO production *in vitro*. Addition of AG to incubation medium shuts down synthesis of NO and inhibits cytolytic activity of these cells.

Production of IL-1β, TNF-α and IL-12 by Mφ after single WBI of BALB/c mice





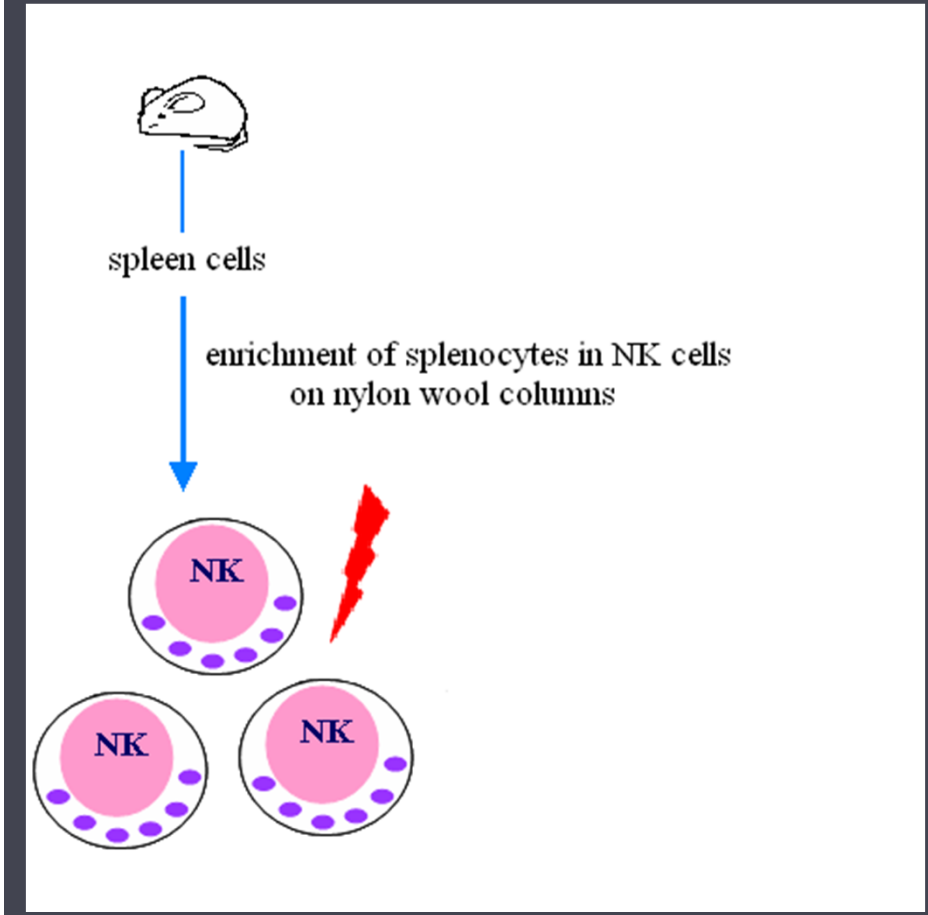


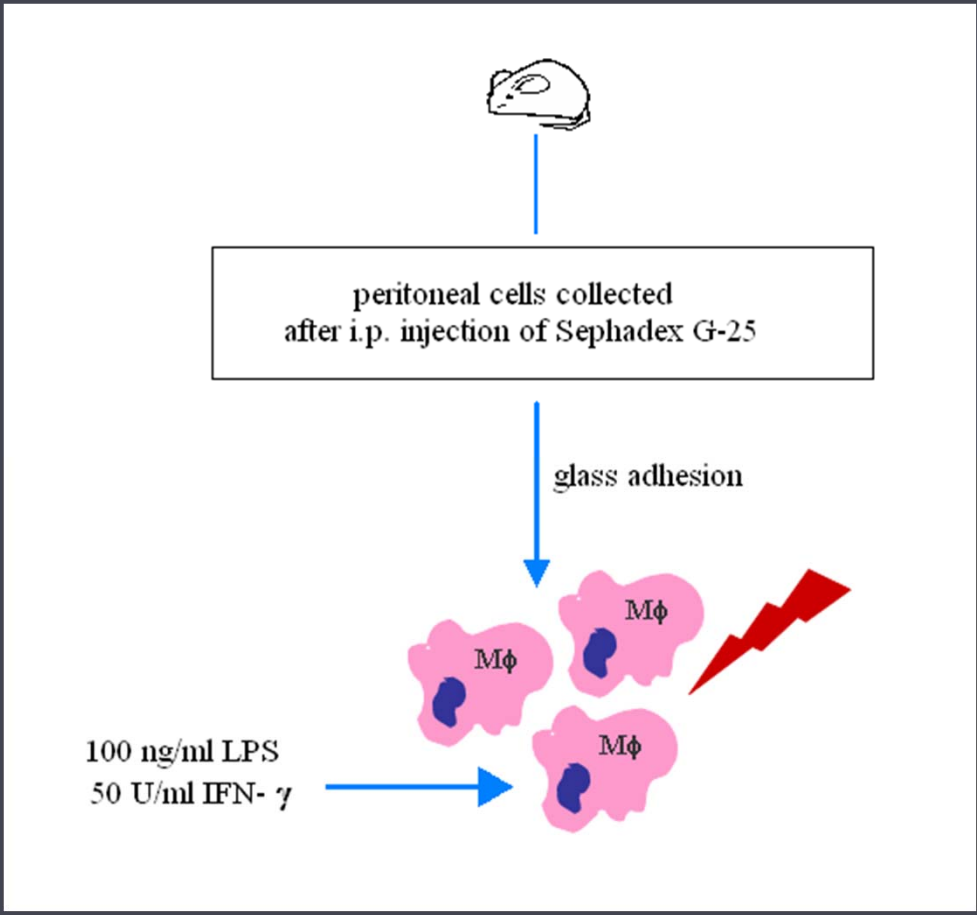
Exposures of mice to 0.1, 0.2, or 1.0 Gy of single X-rays stimulate production of IL-1ß, TNF-α and IL-12 by Mφ.

Anti-tumor activity of macrophages after single WBI of C57BL/6 mice

- Stimulated production of NO by the IFN-γ- and LPS-treated peritoneal Mφ collected on the day of WBI of mice at 0.04 Gy γ-rays; the effect associated with significant enhancement of the cytotoxic function of Mφ *(Ibuki and Goto 1995)*;
- Elevated transcriptional levels of mRNA for both IL-1β and TNF-α in peritoneal Mφ collected from the mice implanted with the Lewis lung carcinoma cells and exposed to 0.075 Gy of X-rays *(Zhang et al. 1998)*.

Experimental protocol:
in vitro irradiation of NK cells and Mφ





Activity of NK cells and Mφ after *in vitro* irradiation

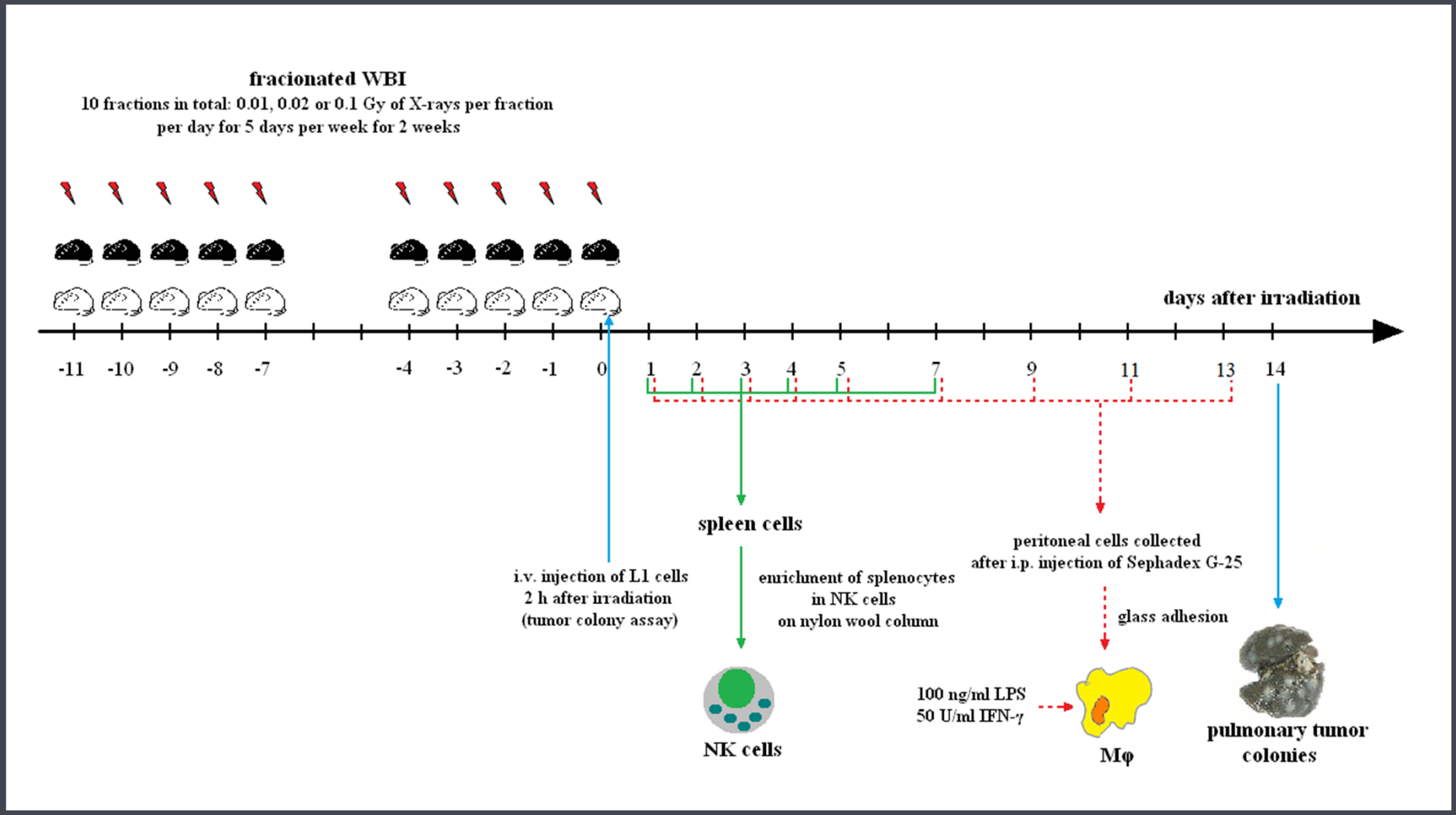
Groups	NK cells		Mφ	
	Cytotoxicity [%]	IFN-γ [pg/ml]	Cytotoxicity [%]	NO_2^- [μM/L]
C	6.0 ± 1.4	46.1 ± 4.0	25.2 ± 3.0	12.5 ± 1.6
0.1 Gy	5.5 ± 1.3	51.3 ± 5.4	24.6 ± 2.9	11.8 ± 1.3
0.2 Gy	5.8 ± 1.5	53.7 ± 5.7	24.9 ± 2.8	13.1 ± 1.5
1.0 Gy	5.8 ± 1.2	44.4 ± 4.2	25.4 ± 3.4	13.1 ± 1.4

NK cell- and Mφ-mediated functions are not significantly affected by irradiations of these cells *in vitro* .

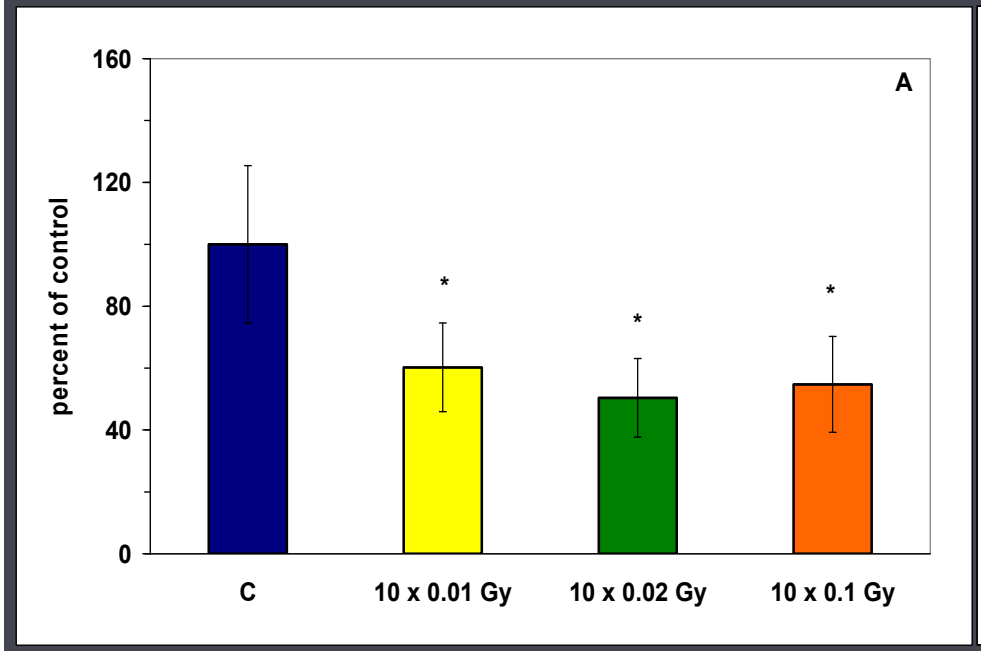
Summary 1

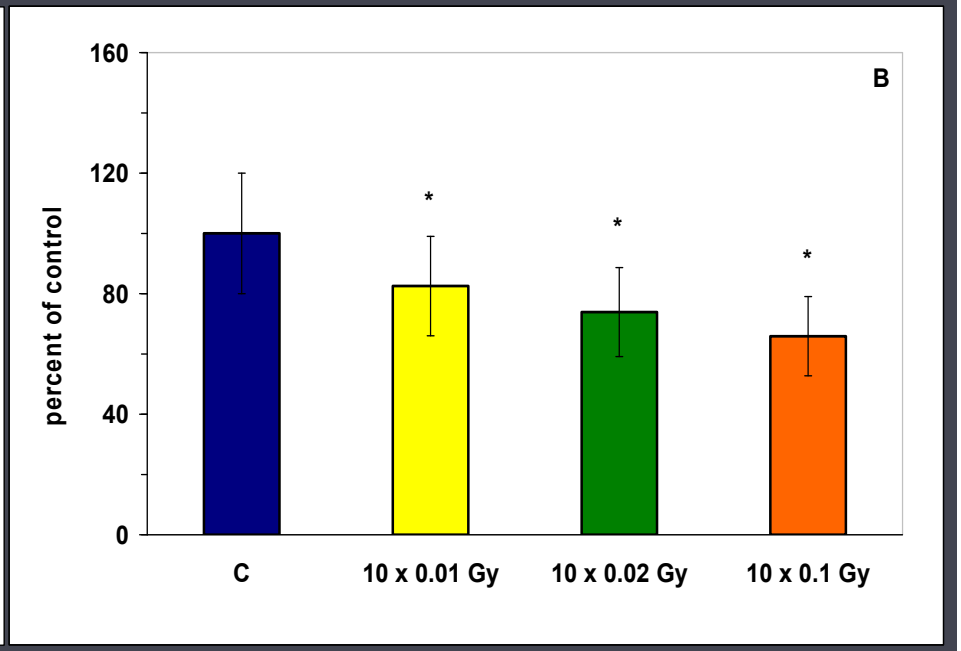
- Suppression of pulmonary tumor colonies by single irradiations of mice at 0.1 or 0.2 Gy X-rays may result from stimulation of defense reactions mediated by NK lymphocytes and/or cytotoxic Mφ;
- Boosting of the cytolytic functions of these anti-tumor effectors by low-level exposures to X-rays requires cooperation of other cells and their products which occur *in vivo* but not *in vitro.*

Experimental protocol: fractionated irradiation



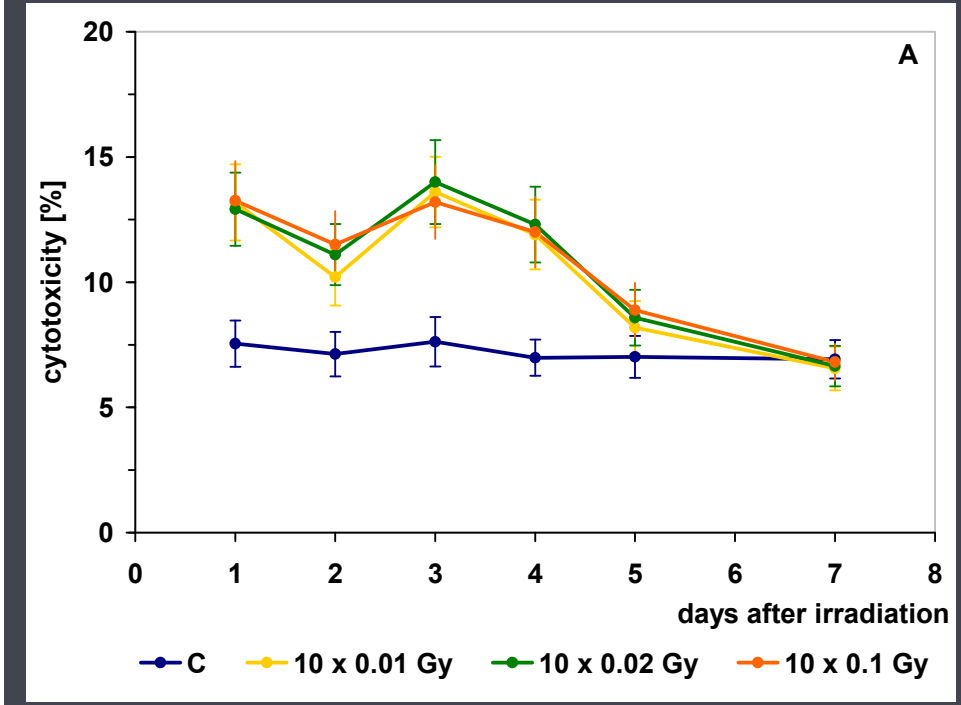
Development of induced metastases in the lungs after fractionated WBI of BALB/c (A) and C57BL/6 (B) mice

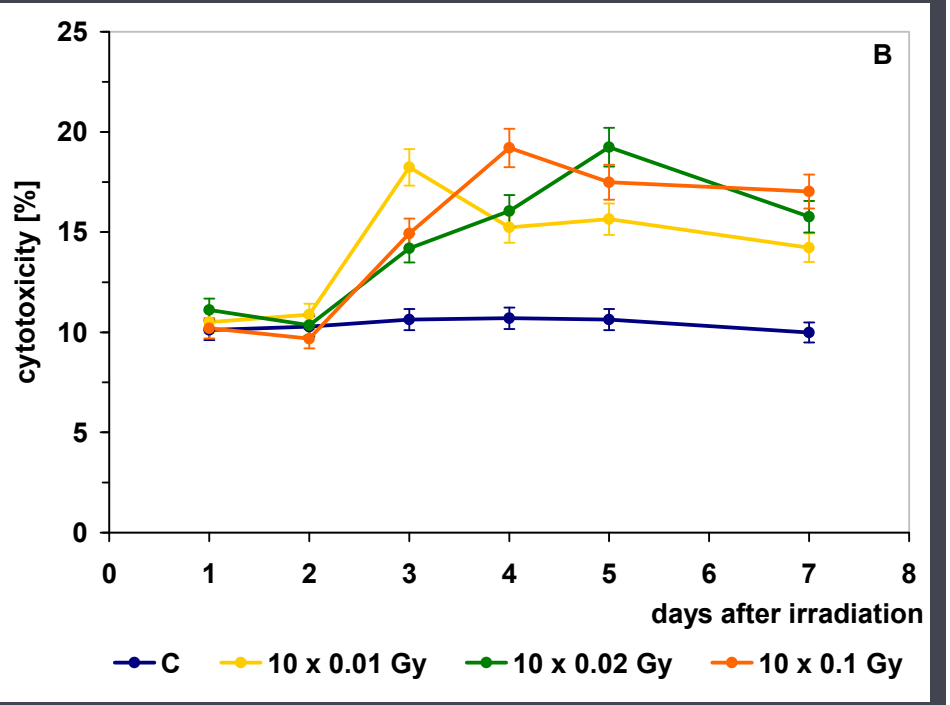




Fractionated WBI with 0.1, 0.2, or 1.0 Gy total doses of X-rays retard development of pulmonary tumor colonies in both strains; the effect is dose-independent in BALB/c and dose-dependent in C57BL/6 mice.

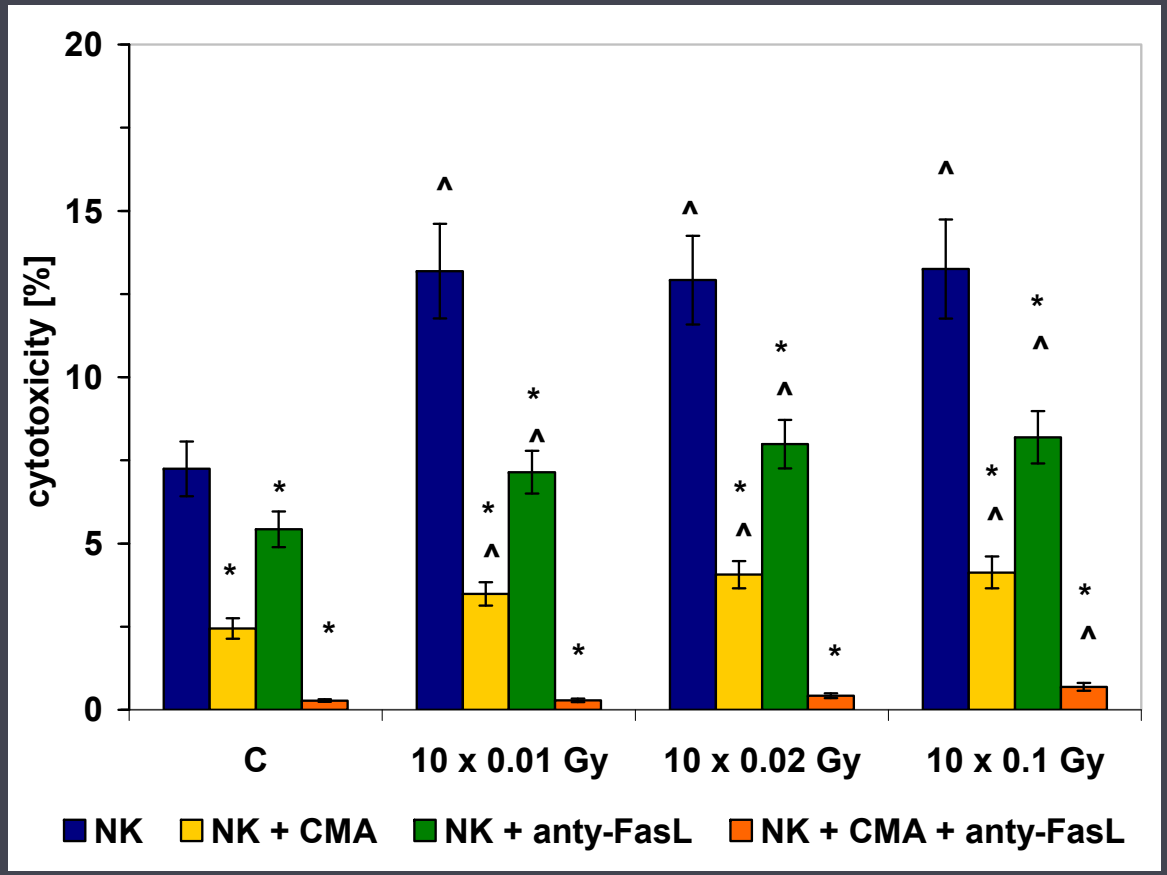
Cytotoxic activity of NK cells after fractionated WBI of BALB/c (A) and C57BL/6 (B) mice





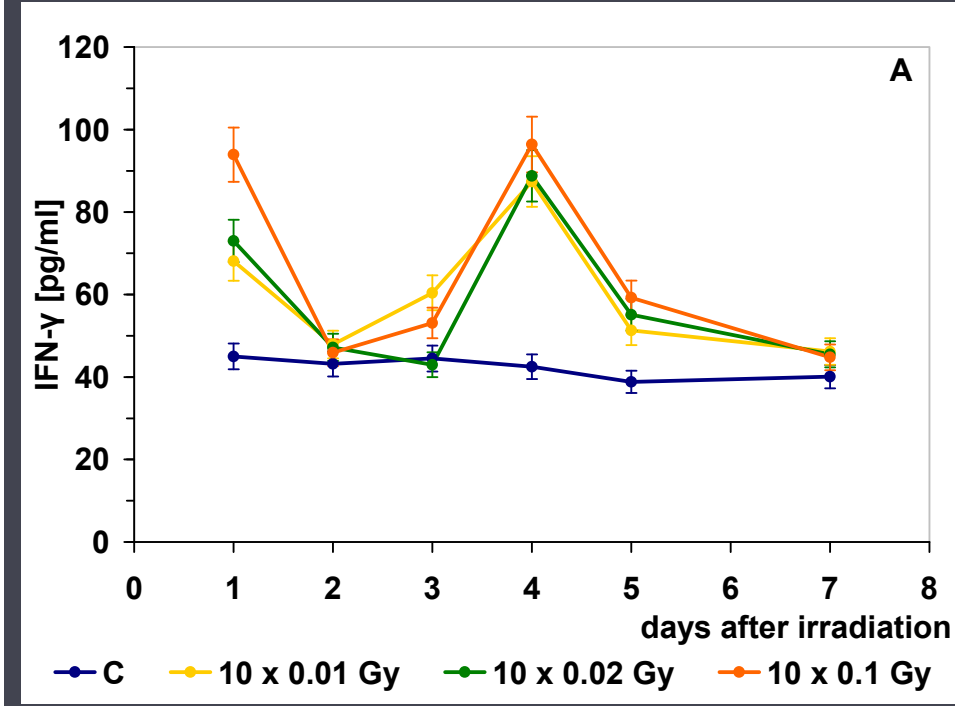
Cytotoxic function of NK cells is significantly stimulated after fractionated WBI of mice at 0.1, 0.2, or 1.0 Gy total doses of X-rays.

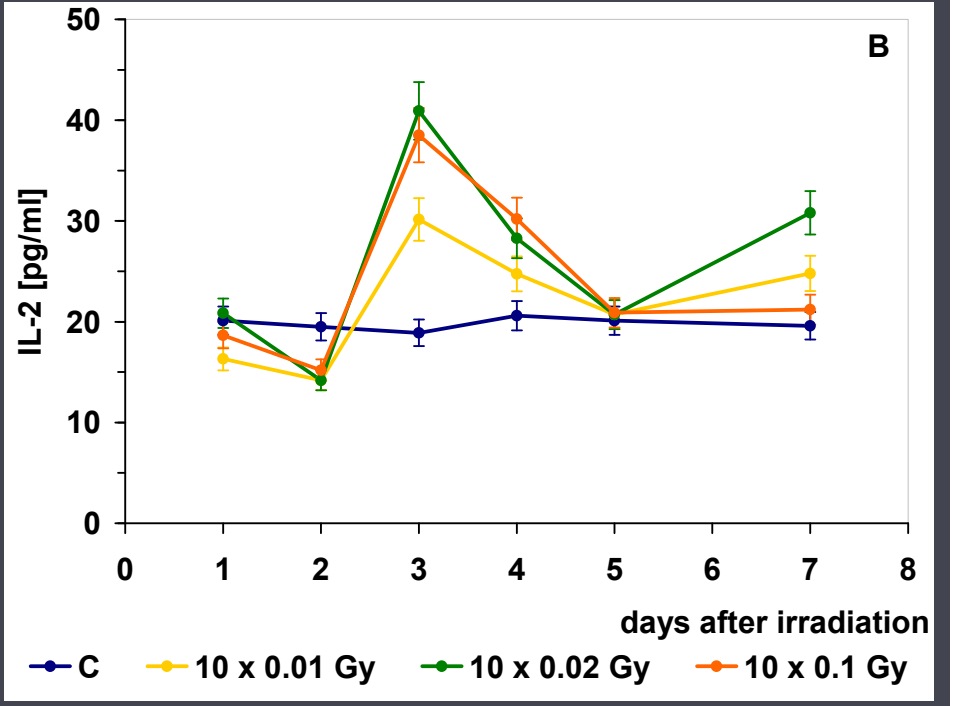
Inhibition of cytotoxic activity of NK cells after fractionated WBI of BALB/c mice



The elevated cytotoxicity of NK cells after fractionated WBI of mice at 0.1, 0.2, or 1.0 Gy total doses of X-rays is mediated by perforin and FasL.

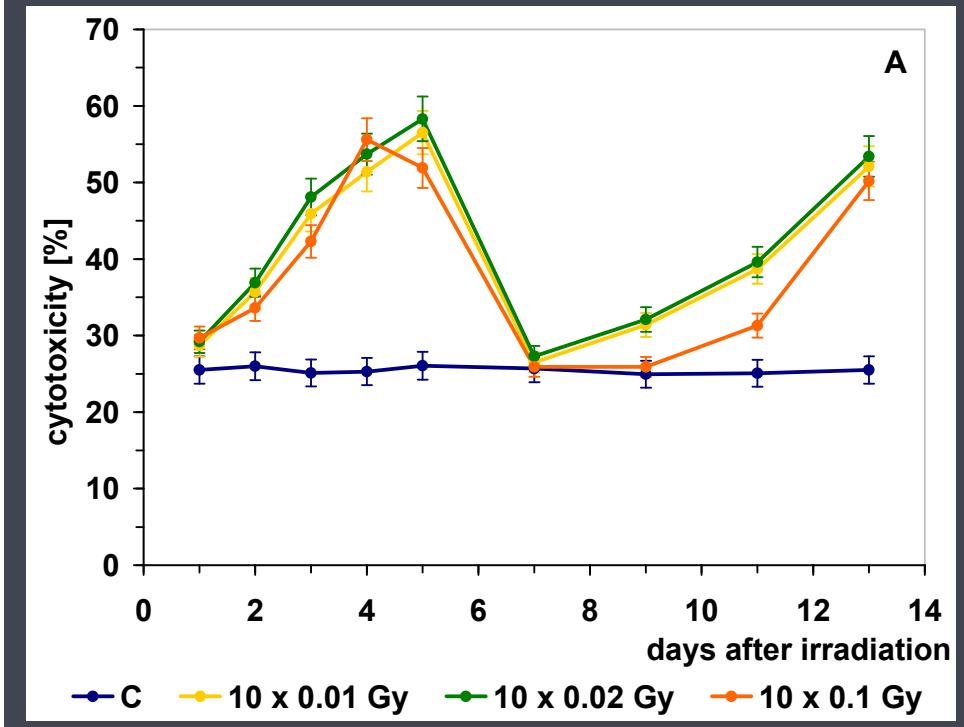
Production of IFN-γ by NK cell-enriched splenocytes (A) and IL-2 by splenocytes (B) after fractionated WBI of BALB/c mice

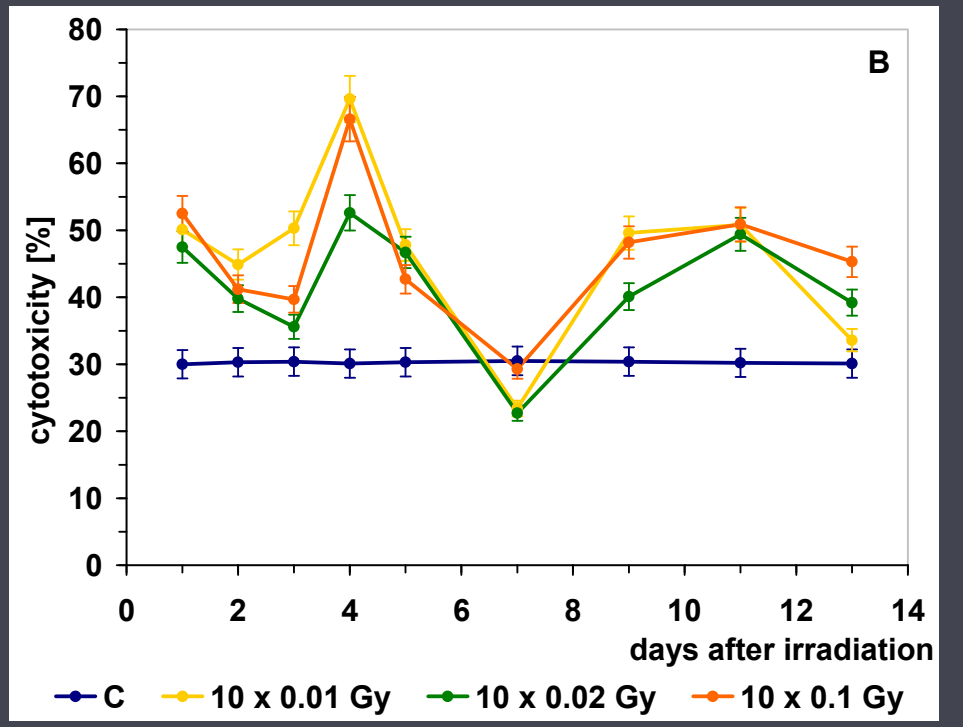




Fractionated WBI of mice at 0.1, 0.2, or 1.0 Gy total doses X-rays stimulates production of IFN-γ by NK cell-enriched splenocytes and IL-2 by total splenocytes.

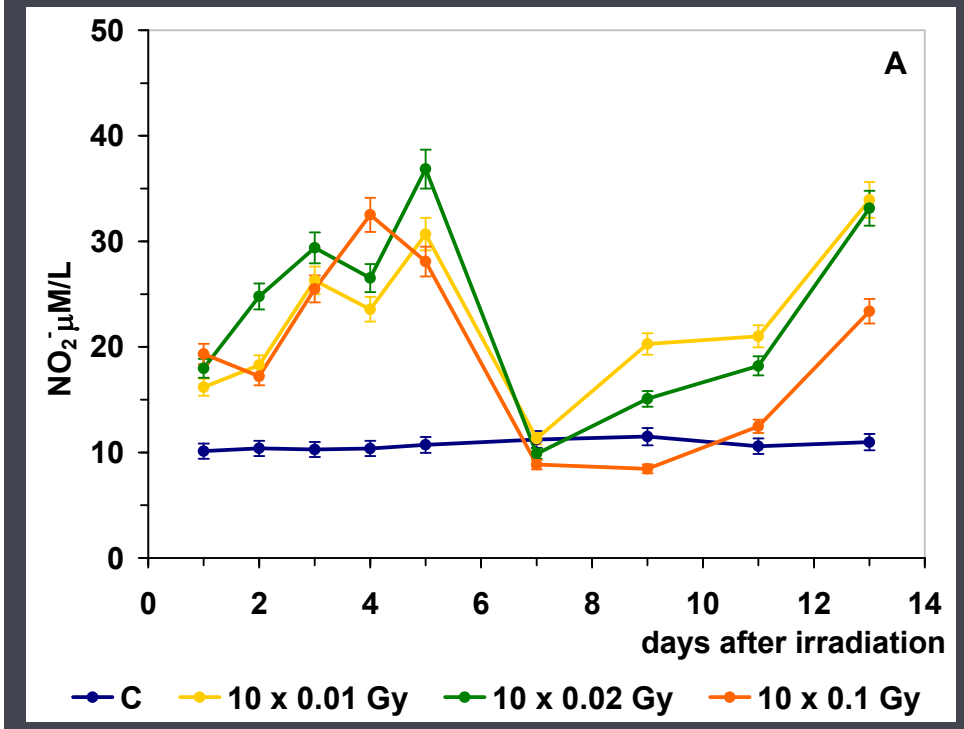
Cytotoxic activity of Mφ after fractionated WBI of BALB/c (A) or C57BL/6 (B) mice

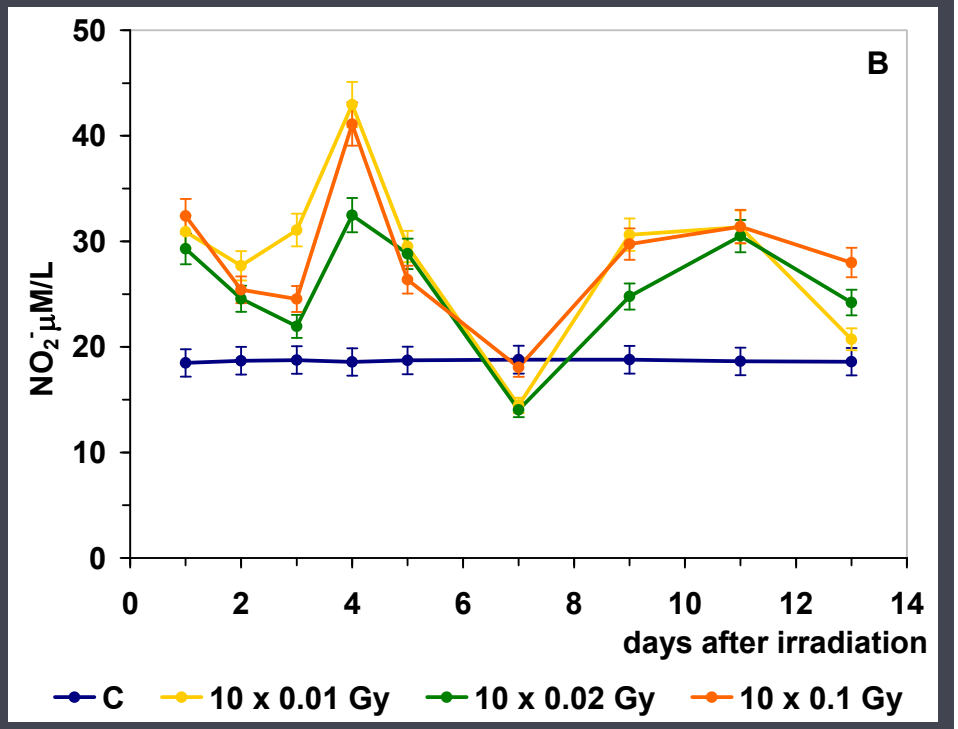




Fractionated exposures of BALB/c and C57BL/6 mice to ten daily doses of 0.01, 0.02 or 0.1 Gy X-rays significantly stimulate Mφ-mediated cytolysis of tumor cells; the effect is biphasic in both strains.

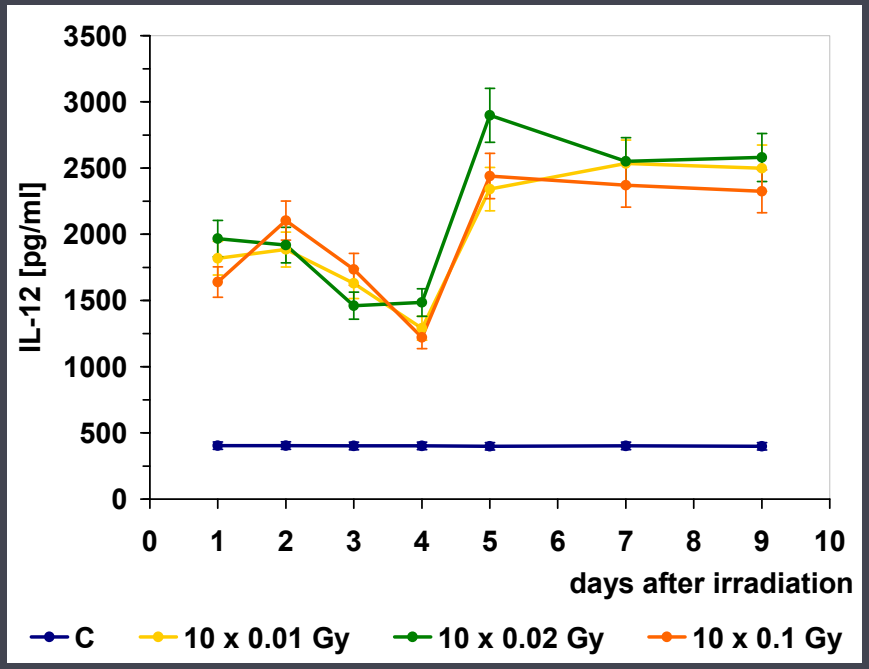
Production of NO by Mφ after fractionated WBI of BALB/c (A) or C57BL/6 (B) mice

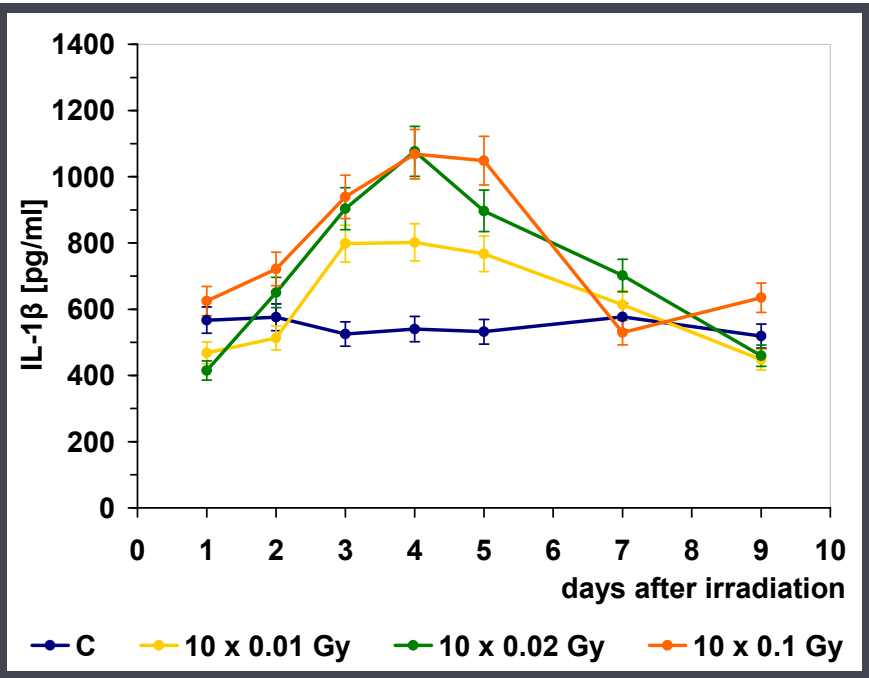


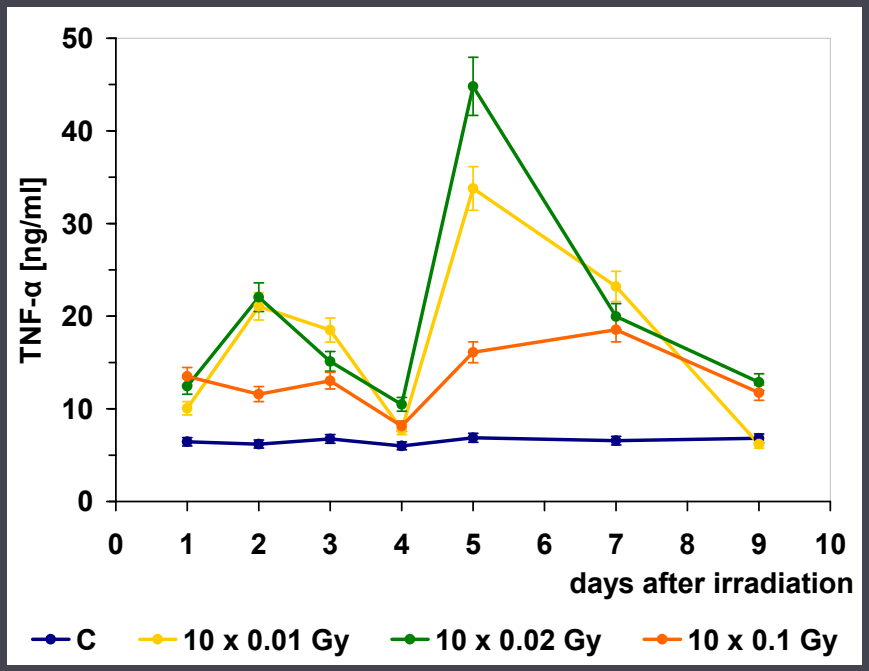


Fractionated exposures of BALB/c and C57BL/6 mice to ten daily doses of 0.01, 0.02 or 0.1 Gy X-rays significantly stimulate production of NO by peritoneal Mφ; the effect is biphasic in both strains.

Production of IL-1β, TNF-α and IL-12 by Mφ after fractionated WBI of BALB/c mice







Exposures of BALB/c mice to ten daily doses of 0.01, 0.02 or 0.1 Gy X-rays stimulate production of IL-1ß, TNF-α, and IL-12 by Mφ.

Summary 2

- Despite some differences between radiosensitive BALB/c and radioresistant C57BL/6 mice in the NK cell- and macrophage-mediated responses to repeated low-level irradiations with 0.01, 0.02 or 0.1 Gy X-rays, the final tumor-inhibitory effects of such exposures were comparable in the two strains.
- Similar anti-metastatic immune mechanisms may operate in the irradiated BALB/c and C57BL/6 mice.
- Whether or not these observations relate to the higher tumor proneness of the irradiated BALB/c compared to C57Bl/6 mice and/or to the different M1/M2 phenotypes of the two strains awaits clarification in future studies.

New grant no. UMO-2011/01/D/NZ7/05389 from the Polish National Science Centre:

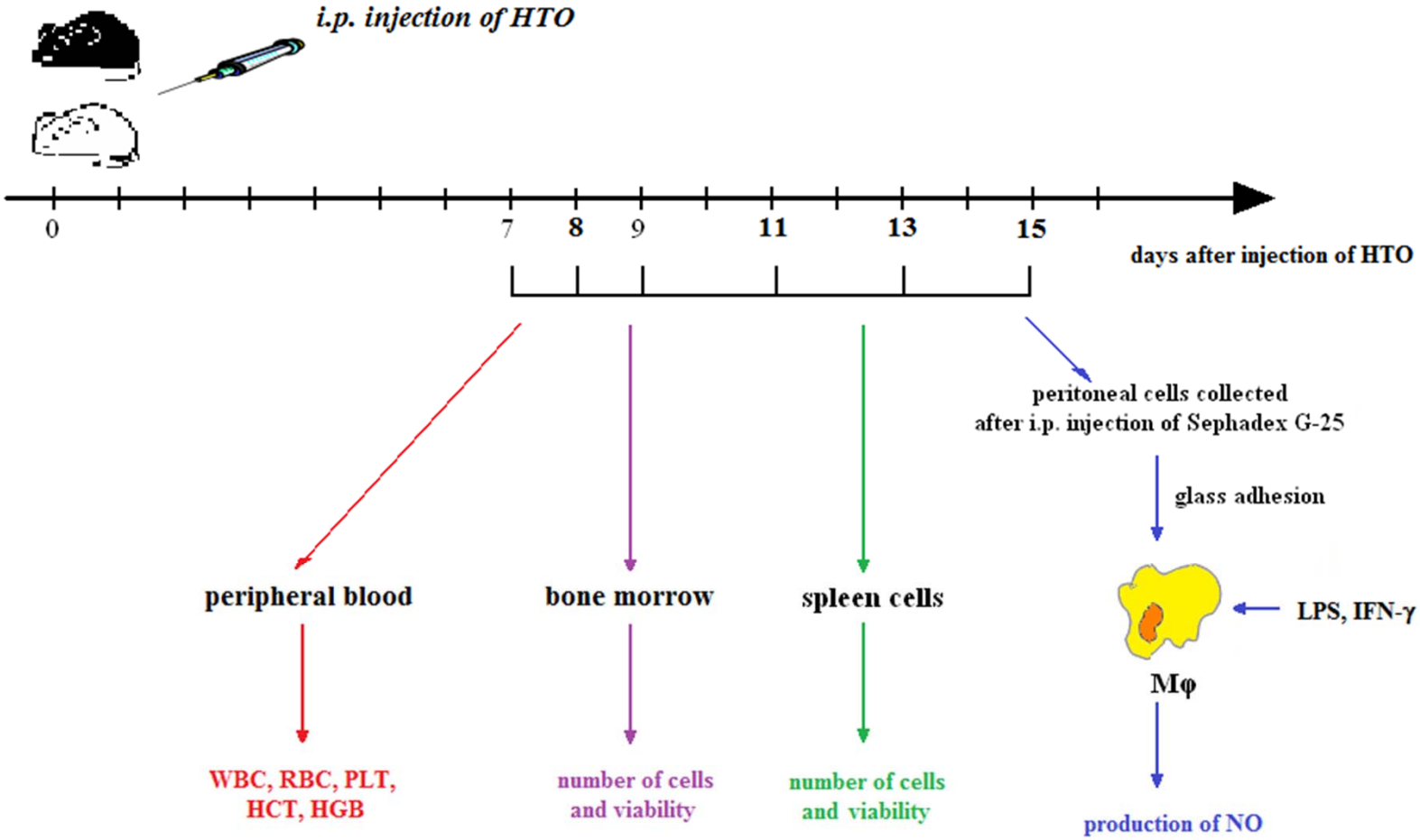
Effect of internal contamination with tritiated water on the innate anti-tumor and inflammatory reactions in radioresistant and radiosensitive mice

Aims:

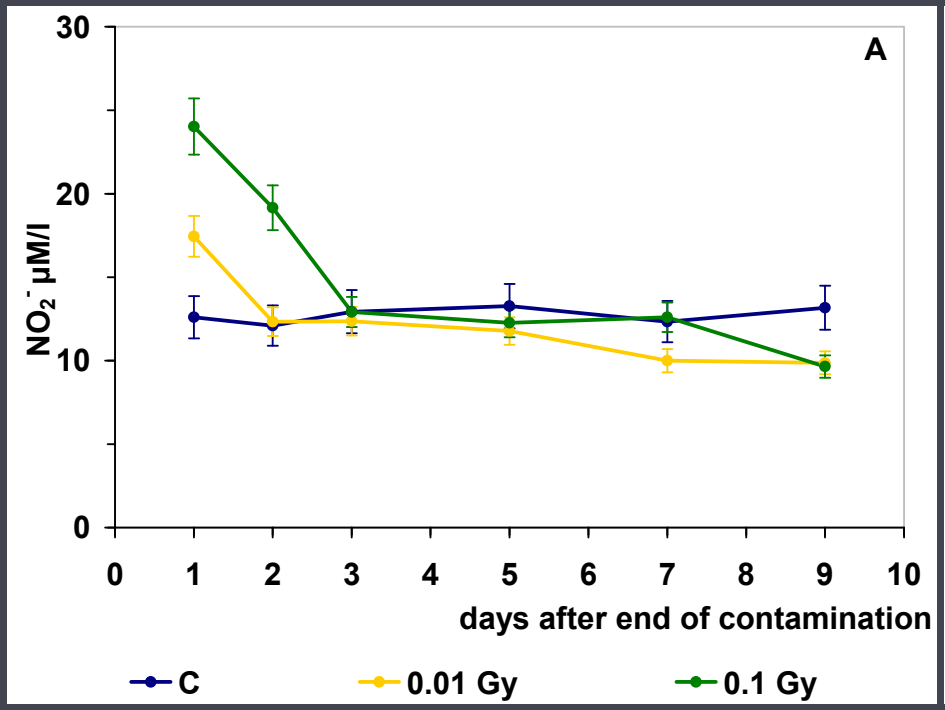
1. Does internal deposition of tritiated water modify development of pulmonary tumor metastases in mice?

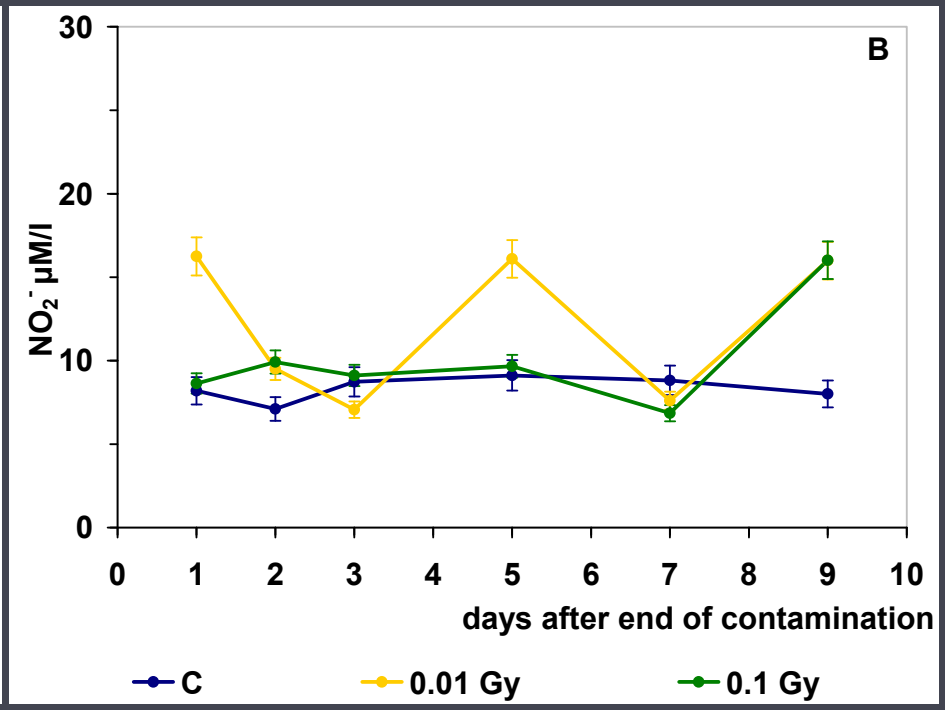
2. Can the effects be associated with functions of activated macrophages and/or NK lymphocytes?

Experimental protocol: internal contamination with HTO



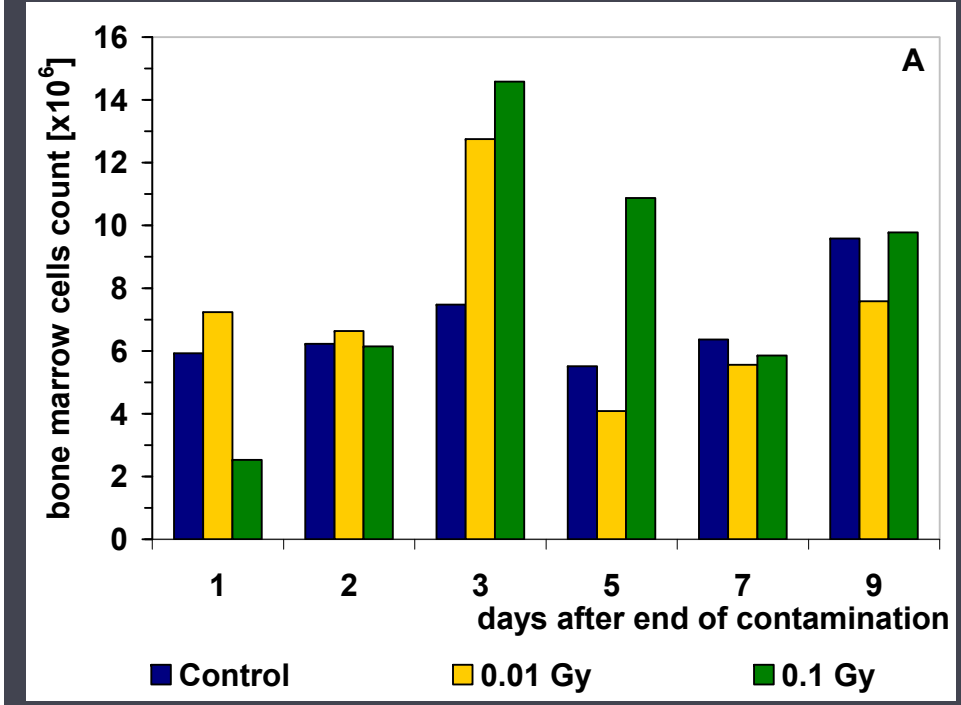
Production of NO by Mφ of BALB/c (A) or C57BL/6 (B) mice contaminated with HTO

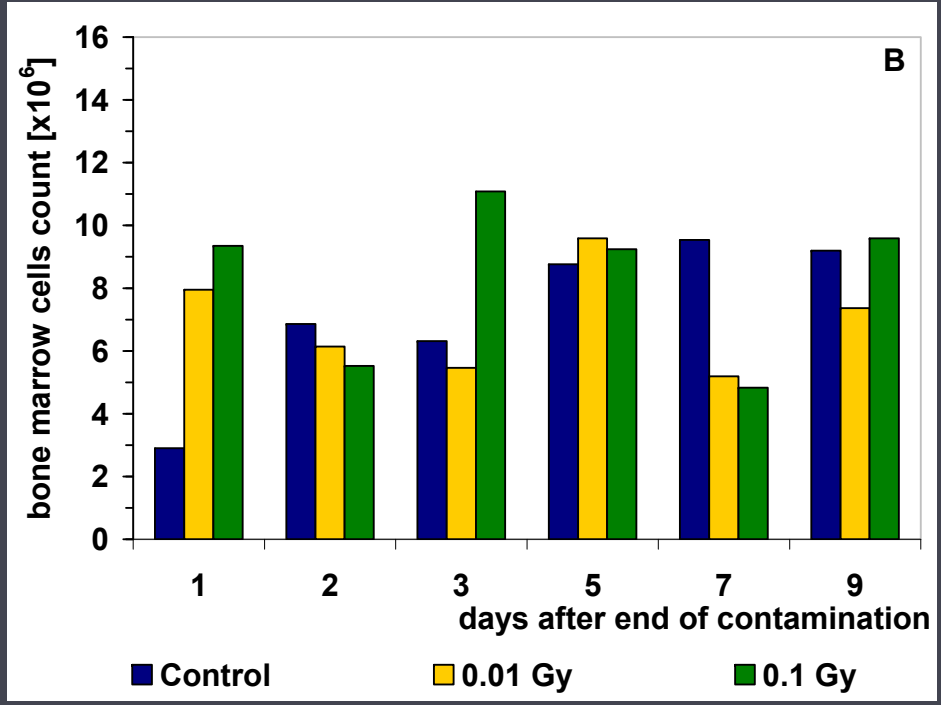




Exposure of BALB/c and C57BL/6 mice to HTO differently stimulate production of NO by peritoneal Mφ; the effect is most pronounced after absorption of 0.1 Gy in the former, and 0.01 Gy in the latter strain.

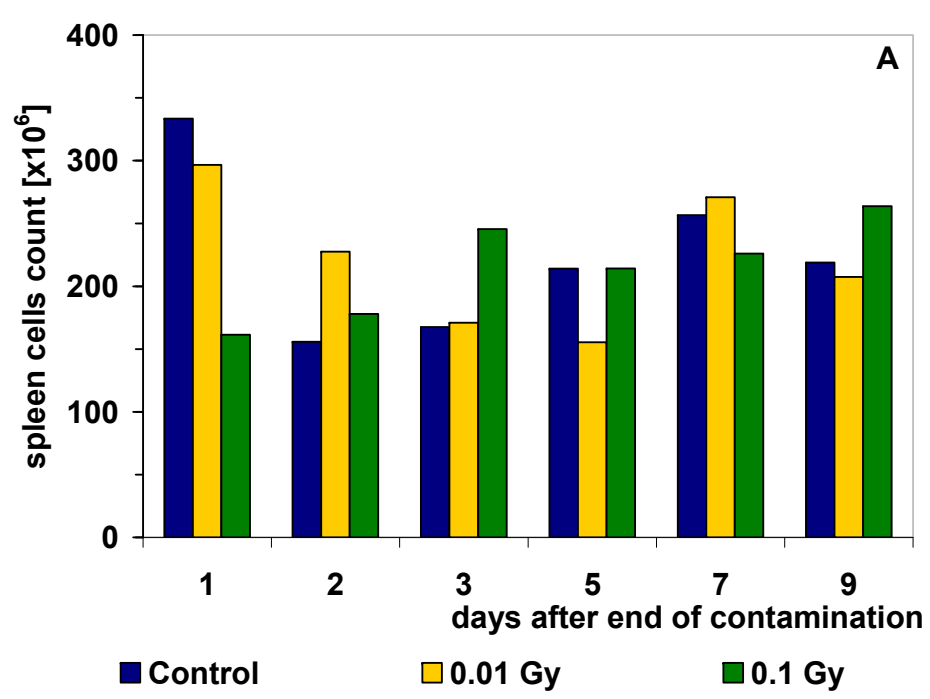
Bone marrow cells counts of BALB/c (A) and C57BL/6 (B) mice after contamination with HTO

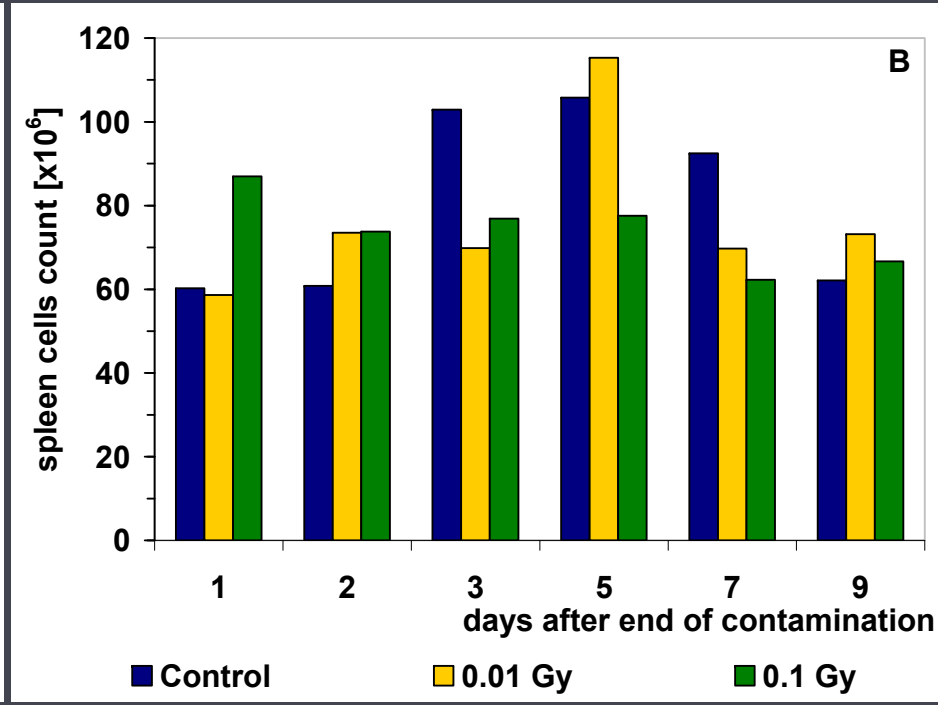




Exposure of BALB/c and C57BL/6 mice to HTO at 0.01 or 0.1 Gy total doses seems to not affect bone marrow cells counts, which are comparable in both strains.

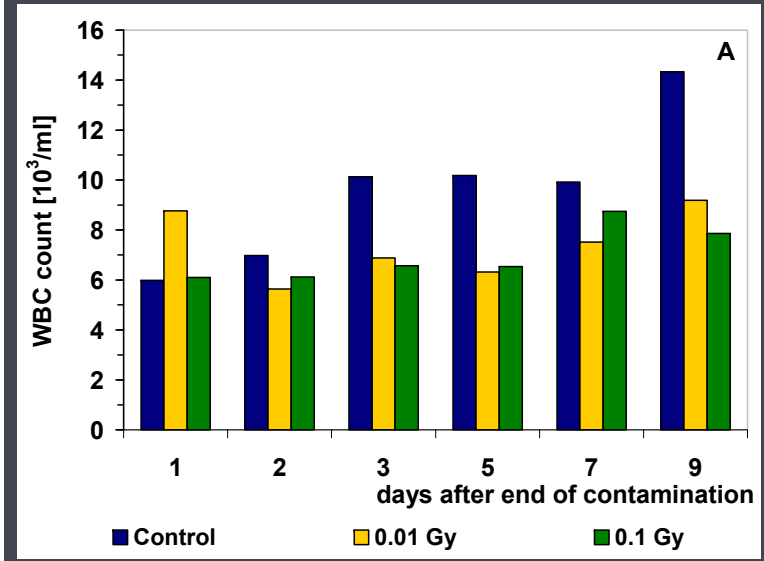
Spleen cells counts in BALB/c (A) and C57BL/6 (B) mice after contamination with HTO

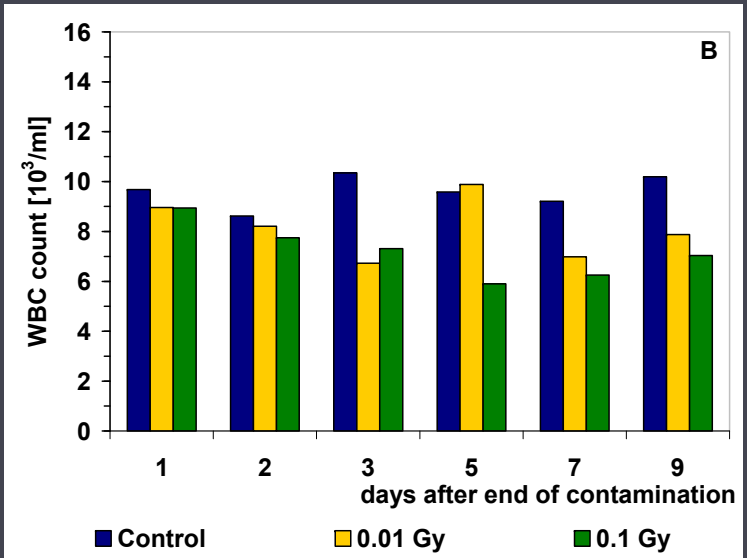




Exposure of BALB/c and C57BL/6 mice to HTO at 0.01 or 0.1 Gy total doses seems to not affect spleen cells counts; the count is about twice as high in the former than in the latter strain.

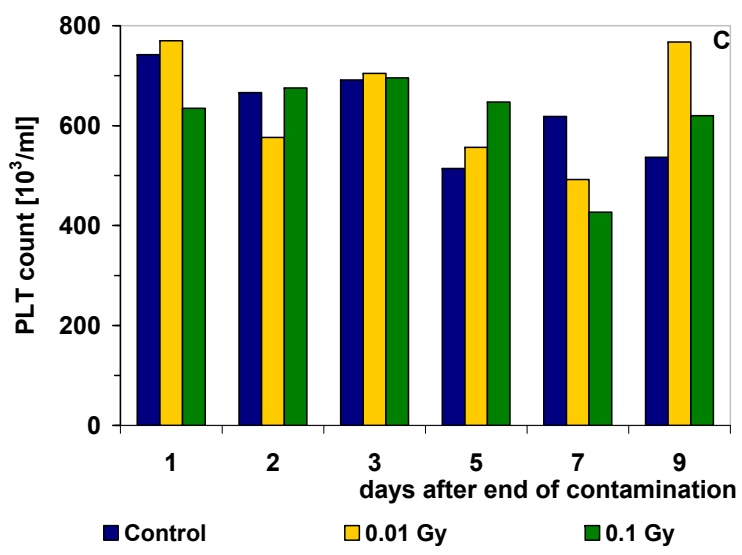
WBC (A,B) and PLT (C,D) counts in BALB/c (A,C) and C57BL/6 (B,D) mice after contamination with HTO

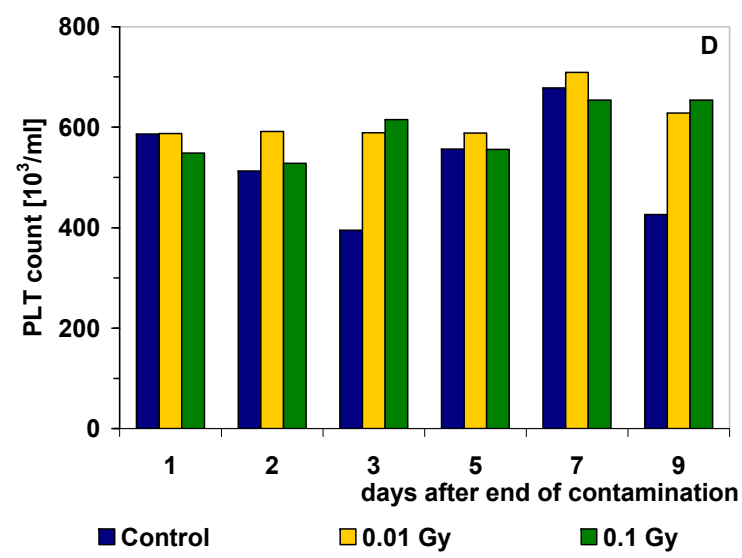




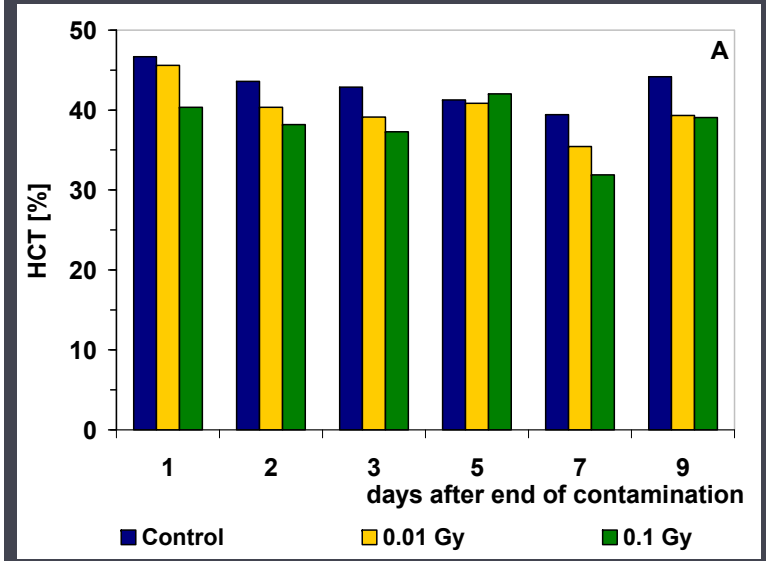
Exposure of BALB/c and C57BL/6 mice to HTO at 0.01 or 0.1 Gy total doses does not affect WBC or PLT counts.

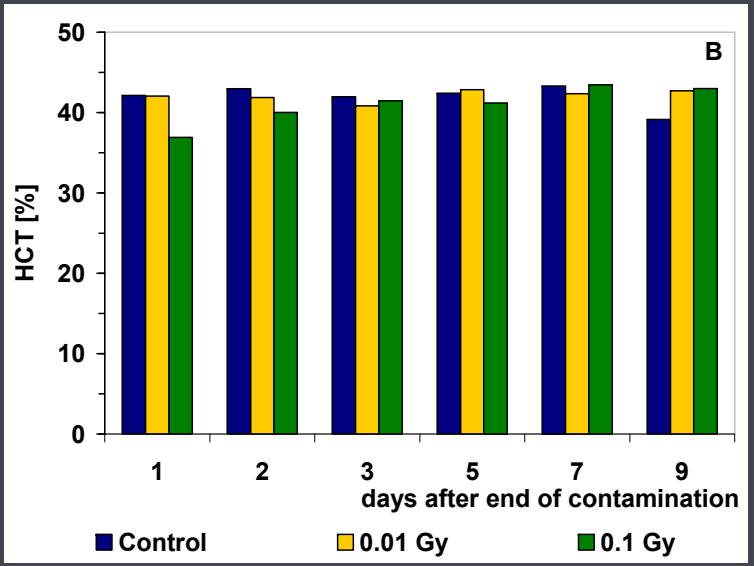
The counts are comparable in both strains.





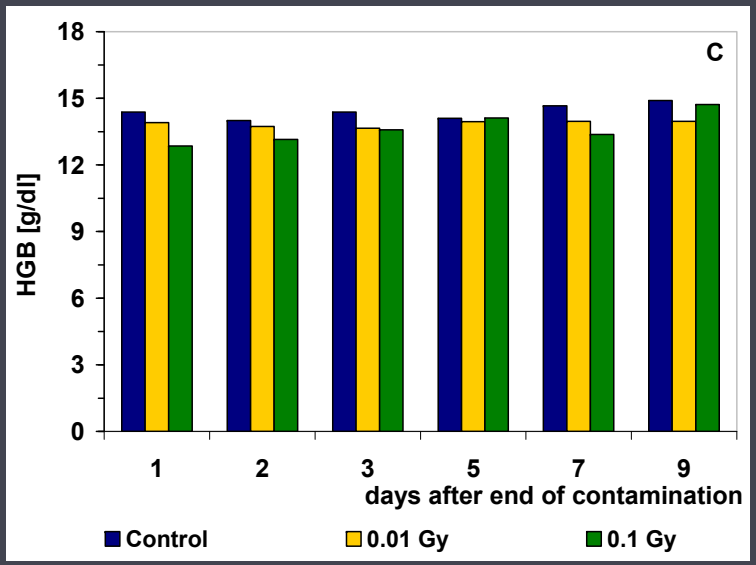
HCT (A,B) and HGB concentration (C,D) in BALB/c (A,C) and C57BL/6 (B,D) mice after contamination with HTO

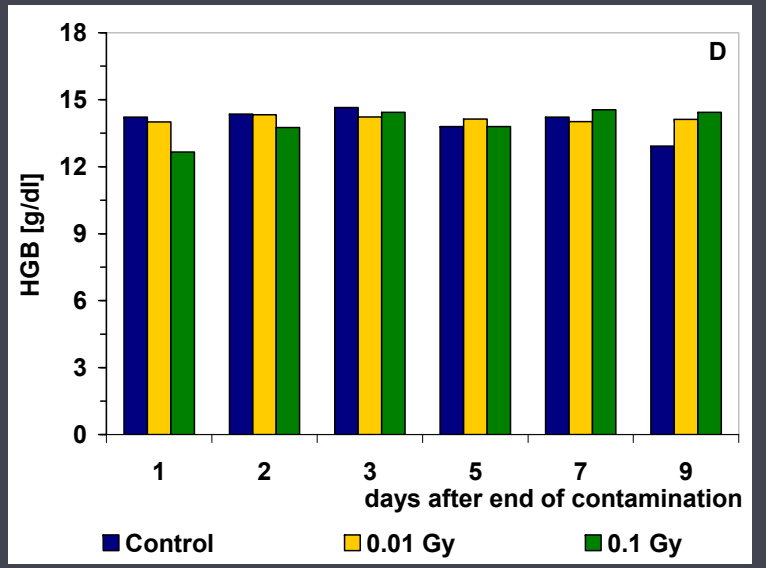




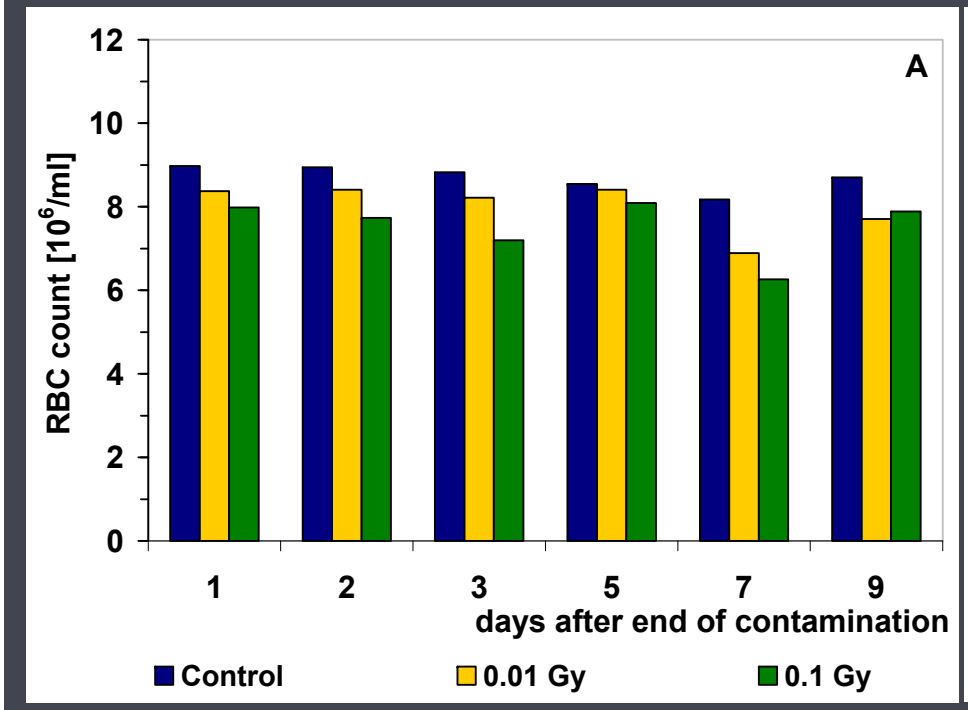
Exposure of BALB/c and C57BL/6 mice to HTO at 0.01 or 0.1 Gy total doses does not affect HCT or HGB.

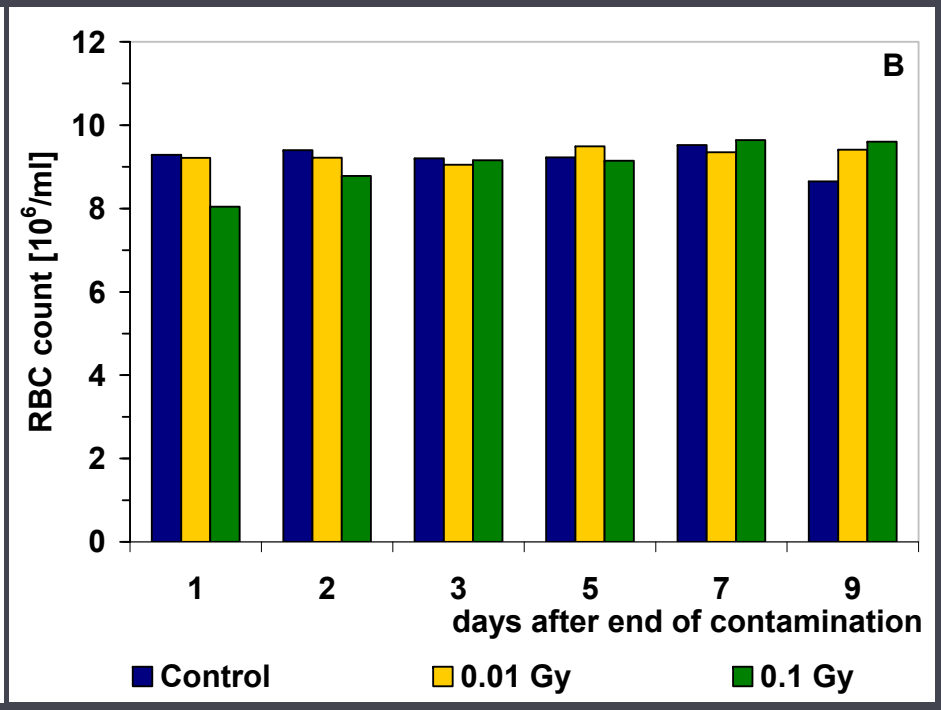
The results are comparable in both strains.





RBC counts in BALB/c (A) and C57BL/6 (B) mice after contamination with HTO





Exposure of BALB/c and C57BL/6 mice to HTO at 0.01 or 0.1 Gy total doses does not affect RBC counts which are comparable in both strains.

Summary 3

- Internal contamination of BALB/c and C57BL/6 mice with HTO at 0.01 or 0.1 Gy total doses do not affect bone marrow and spleen cells' numbers, peripheral blood cell counts, or blood HGB and HCT;
- NO production in macrophages obtained from HTO-contaminated BALB/c mice is likely to be different from that in counterpart cells obtained from HTO-contaminated C57BL/6 mice.

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