Irradiation of Genetically Modified Plasmacytoma Vaccines Results in Upregulation of CD80 Molecule Expression, IL-2 Production and Higher Therapeutic Efficacy of the Vaccines

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Abstract. It has been found previously that irradiated, IL-2 gene-modified plasmacytoma (X63-m-IL-2) vaccines are more efficient in the therapy of the parental (X63-Ag8.653) plasmacytoma than live plasmacytoma vaccines. In this communication, we have demonstrated that irradiation of murine IL-2-producing plasmacytoma vaccines resulted in upregulation of CD80 molecule expression and IL-2 production. The expression of MHC class I antigens was not altered. The upregulation of the CD80 membrane molecule expression in X63-m-IL-2 cells was higher after irradiation with 150 Gy than after irradiation with 50 Gy. Comparable upregulation of the CD80 molecule expression has also been demonstrated after irradiation of the parental murine X63-Ag8.653 plasmacytoma cells. The results indicate that upregulation of the CD80 molecule expression and enhanced IL-2 production in irradiated X63-m-IL-2 cells was responsible for the higher therapeutic effectiveness of the irradiated plasmacytoma vaccine.

To induce efficient antitumour immune responses, several signals are required. The first, activating signal, is based on the recognition of tumour rejection antigen (TRA) by the corresponding receptors expressed on the surface of immune cells, i.e. the corresponding T-cell receptor (TCR) interacts with antigen presented in the context with major histocompatibility complex (MHC) class I molecules. The second, synergistically acting activating signal, is a co-stimulatory signal provided by the interaction of CD80 molecules expressed on antigen presenting cells with the corresponding ligands expressed on lymphocytes. A subsequent expansion of tumour-specific activated effector cells, which is required for a quantitatively sufficient immune response, is mediated by mitogenic signals, primarily by IL-2 (for a review, see Townsend and Bodmer, 1989; Germain, 1994; Bubeník, 1996).

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Abbreviations: IL-2 – interleukin 2, mAb – monoclonal antibody, MHC – major histocompatibility complex, TRA – tumour rejection antigen, TCR – T-cell receptor.

It has been demonstrated previously that irradiated, IL-2 gene-modified plasmacytoma vaccines were more effective in local therapy of parental murine plasmacytoma than live plasmacytoma vaccines (Šímová et al., 1998). The aim of this communication was to examine the mechanism of the higher therapeutic efficacy of irradiated, IL-2 gene-modified plasmacytoma vaccine in comparison with the non-irradiated plasmacytoma vaccine. The experiments were designed to investigate changes, caused by irradiation, in the level of both, activating and mitogenic signals.

Material and Methods

Cell lines

Murine myeloma cell line X63-Ag8.653 of BALB/c origin (Kearney et al., 1979) and X63-m-IL-2 cell line obtained by transformation of X63-Ag8.653 cells with murine IL-2 cDNA and constitutively secreting recombinant IL-2 (Karasuyama and Melchers, 1988) were kindly provided by Dr F. Melchers, Basel Institute for Immunology. The characteristics and tumorigenicity of the cell lines were described earlier (Karasuyama and Melchers, 1988; Bubeník et al., 1990). Murine cell line MC12-CD80, obtained by transfection of 3-methylcholanthrene-induced fibrosarcoma with murine CD80 cDNA and expressing CD80 (Rössner et al., 1997), was also used as a control. Tumour cell lines were maintained in RPMI 1640 medium (Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Prague, Czech Republic) supplemented with 10% foetal calf serum (BioClot, Ltd., Aidenbach, Switzerland), L-glutamine (Sevac, Prague, Czech Republic), penicilline (Biotika, Slovenská Lupča, Slovakia), streptomycine (Sigma, Steinheim, Germany), 2-mercaptoethanol (Calbiochem, La Jolla, CA) and incubated at 37° C in a humidified atmosphere with 5% CO_2. For experiments, the X63-Ag8.653 and the X63-m-IL-2 cells were irradiated with a dose of 50 or 150 Gy.

Vaccination of tumour-bearing mice

BALB/c male mice, 2–4 months old, were obtained from the Anlab Co., Prague, Czech Republic. Mice were inoculated i.p. on day 0 with a dose of 1×10^5 X63-Ag8.653 plasmacytoma cells and randomized into experimental and control groups. Experimental groups were vaccinated

i.p. on day 3 with a dose of 2.5×10^7 genetically modified X63-m-IL-2 cells irradiated with a dose of 50 Gy, or live non-irradiated X63-m-IL-2 cells. The differences between survival of experimental and control mice were compared by Student's t-test, and the significance of differences between tumour takes in experimental and control groups was estimated using χ^2 test.

Flow cytometry

Surface expression of the CD80 molecule on irradiated and non-irradiated cells was determined by staining with specific FITC-conjugated anti-CD80 monoclonal antibody (mAb). The cells were washed, resuspended in phosphate-buffered saline containing 1% bovine serum albumin and 0.02% NaN_3, and directly labelled with anti-CD80 mAb (FITC anti-mouse CD80, clone 16-10A1, Pharmingen, San Diego, CA; 1 µg per 5×10^5 cells, 45 min incubation at 4°C). FACS analysis was performed using ELITE FACScan flow cytometer (Coulter, Miami, FL). The cell line MC12-CD80 expressing the CD80 molecule in 96% of the cell population (Rössner et al., 1997) was used as a positive control. FITC-conjugated anti-CD8 mAb (clone 53-6.7, Sigma-Aldrich, St. Louis, MO) served as a non-specific control antibody and was used under the same conditions. For each measurement, 10^4 cells were employed.

IL-2 determination and MHC class I molecule detection

The amounts of IL-2 in culture supernatants of 50 Gy-irradiated and non-irradiated X63-m-IL-2 plasmacytoma cells were determined after two days of cultivation using the enzyme-linked immunosorbent assay (Genzyme, Cambridge, MA). MHC class I antigen expression was determined by a microcytotoxicity test as described previously (Čapková et al., 1983). The H-2 alloantisera were produced in the Institute of Molecular Genetics, Prague, by immunization of F1 hybrids of inbred and congenic resistant strains by repeated injections of spleen and thymus cell suspension from the donor strain. The specificity of the antisera was determined by testing against a panel of strains with relevant H-2 haplotypes. The H-2 antisera against $H\text{-}2K^d$, $H\text{-}2D^d$, $H\text{-}2K^b$ and $H\text{-}2D^b$ were used. Details are given in the paper Čapková et al. (1990).

Results

Effect of irradiation on the therapeutic efficacy of plasmacytoma vaccines

Local i.p. administration of both, live and 50 Gy-irradiated, IL-2-secreting X63-m-IL-2 cells significantly prolonged the survival of the vaccinated mice. However, only the administration of the irradiated X63-m-IL-2 vaccine significantly reduced (9/15) the percentage of tumour takes as compared to the effect of administration of the live vaccine (Fig. 1).

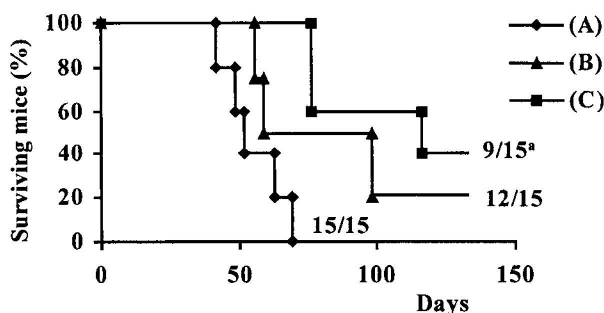

Fig. 1. Effectiveness of local therapy with 50 Gy-irradiated and live, IL-2-secreting X63-m-IL-2 plasmacytoma vaccines. Groups of mice were challenged i. p. on day 0 with 1×10^5 X63-Ag8.653 plasmacytoma cells and vaccinated on day 3 with 2.5×10^7 of the respective vaccine. (A) unvaccinated control mice; (B) mice vaccinated with live X63-m-IL-2 cell vaccine; (C) mice vaccinated with 50 Gy-irradiated X63-m-IL-2 cell vaccine; $^aP > 0.01$ as compared to untreated controls.

Effect of irradiation on the expression of co-stimulatory CD80 molecules

The expression of CD80 molecules in X63-m-IL-2 and X63-Ag8.653 cells was tested by FACS analysis. Cells of both X63-m-IL-2 and X63-Ag8.653 cell lines exhibited low expression of the CD80 molecules. Immediately after irradiation of the cells, no difference between irradiated and live cells was observed (Fig. 2C). Irradiation followed by two-day cultivation of cells increased the membrane expression of CD80 molecules in both X63-m-IL-2 and X63-Ag8.653 cells (Fig. 2A,B). The increase in CD80 expression was higher after irradiation with the dose of 150 Gy than with the dose of 50 Gy. As a positive control, MC12-CD80 cells, constitutively expressing an inserted CD80 molecule, were utilized (Fig. 2D).

Effect of irradiation on IL-2 production and MHC class I antigen expression

To assess IL-2 production in live and irradiated X63-m-IL-2 cells that were used as vaccines in the therapy of murine plasmacytoma X63-Ag8.653, cell supernatants obtained 2 days after irradiation were tested by ELISA. As can be seen in Table 1, *in vitro* production of IL-2 was variable (Exp. 1–5); however, a reproducibly higher production of IL-2 by irradiated cells was determined in five independent experiments performed with the 6th–13th *in vitro* generation of X63-m-IL-2 cells. The live X63-m-IL-2 cells released 3,700–18,480 IU IL-2/ml *in vitro*, whereas comparable numbers of the 50 Gy-irradiated X63-m-IL-2 cells released 11,500–28,500 IU/ml.

The expression of the MHC class I antigen $H\text{-}2D^d$ in X63-m-IL-2 murine genetically modified plasmacytoma cells was determined by the microcytotoxicity assay. Following irradiation of the cells with a dose of 50 Gy and 2-day cultivation, no changes in the expression of the MHC class I antigen $H\text{-}2D^d$ were found in comparison with live X63-m-IL-2 cells (data not shown).

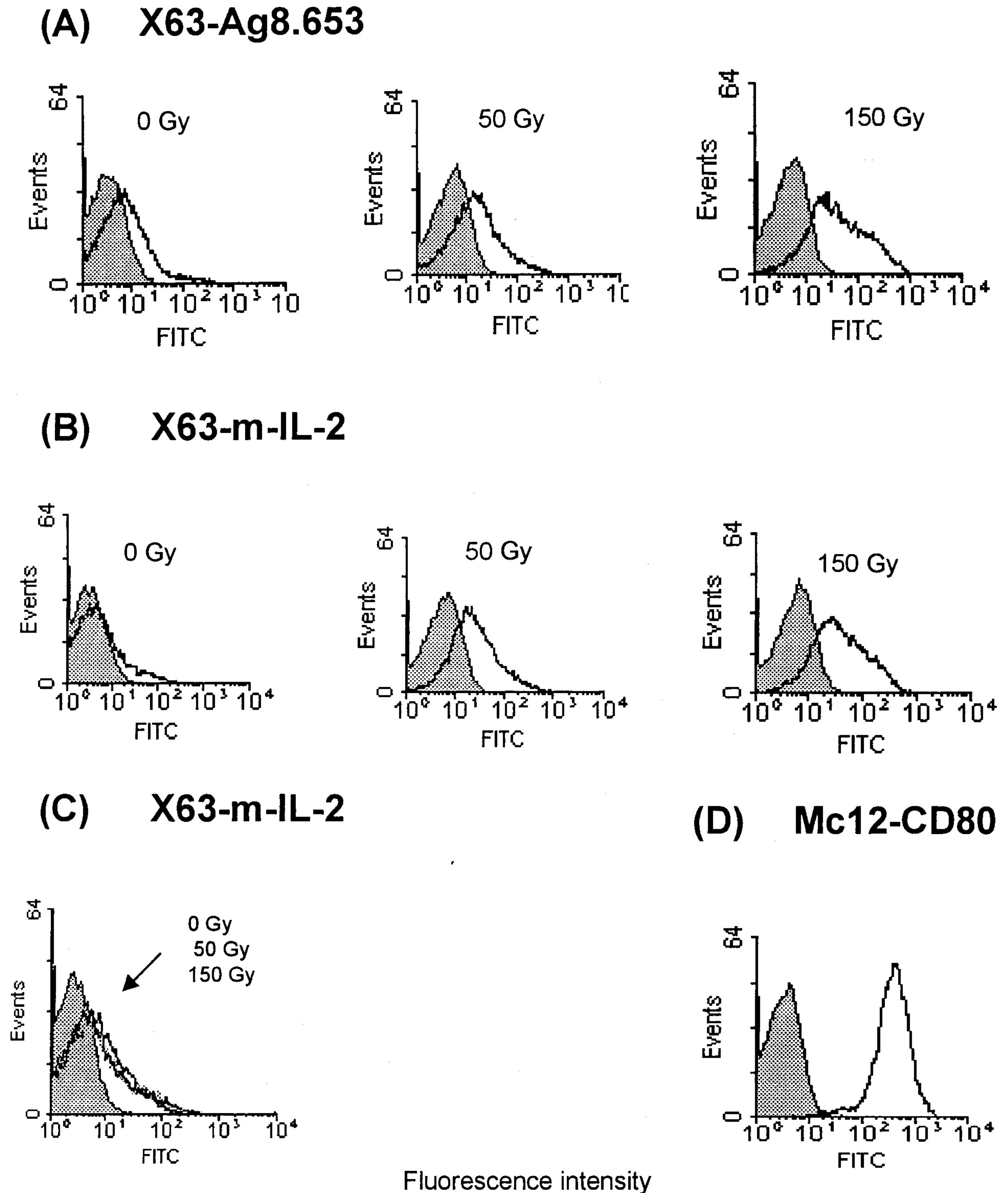

Fig. 2. Effect of irradiation on the expression of CD80 molecules. (A) Expression of CD80 molecules on the X63-Ag8.653 cells, non-irradiated or irradiated with 50 Gy or 150 Gy after 2 days in culture. (B) Expression of CD80 molecules in the X63-m-IL-2 cells non-irradiated and irradiated with 50 Gy or 150 Gy after 2 days in culture. (C) Expression of CD80 molecules in the X63-m-IL-2 cells non-irradiated and irradiated with 50 Gy and 150 Gy 2 h after irradiation. (D) Expression of CD80 molecules on the surface of MC12 sarcoma carrying an inserted CD80 gene (positive control). FITC-conjugated anti-CD8 mAb served as a non-specific control antibody (grey area).

Table 1. Comparison of in vitro IL-2 production by live and irradiated X63-m-IL-2 plasmacytoma vaccines

Exp. No.	*In vitro* generation	Production of IL-2 (IU) after irradiation with a dose of	
		0 Gy	50 Gy
1	6	13500	14100
2	6	12840	14100
3	10	18480	28500
4	12	14520	18300
5	13	3700	11500

Discussion

For safety reasons, the cytokine gene-modified tumour cells used as vaccines in cancer therapy have to be irradiated. In a previous communication we have found that irradiated, IL-2 gene-modified plasmacytoma vaccines were more efficient in parental plasmacytoma therapy than their live counterparts (Šímová et al., 1998). To study the mechanism responsible for the higher therapeutic efficacy of irradiated vaccines, experiments were designed to investigate the expression of IL-2, CD80 and H-2 molecules.

It has been demonstrated that irradiated tumour cells transduced with the IL-2 gene may continue to secrete IL-2 *in vitro* for a substantial period of time (Gansbacher et al., 1992; Porgador et al., 1994; Bubeník, 1996). Increased *in vitro* IL-2 secretion and enhanced expression of IL-2 mRNA after irradiation has been reported in IL-2-transduced murine and human melanomas (Abdel-Wahab et al., 1996). In accordance with the findings of Abdel-Wahab et al., in our experimental model higher levels of secreted IL-2 were found in supernatants of irradiated X63-m-IL-2 cells in comparison to supernatants of live X63-m-IL-2 cells. However, the higher secretion of IL-2 after irradiation of the genetically modified cells was variable, depending apparently on as yet unidentified differences in cultivation conditions.

In several tumour cell systems, an enhanced expression of MHC class I molecules has been described (Saito et al., 1988; Hauser et al., 1993; Klein et al., 1994; Abdel-Wahab et al., 1996). In contrast to these findings, using the microcytotoxicity assay we did not observe any significant increase in the expression of the H-2D^{d} molecule after irradiation of the X63-Ag8.653 plasmacytoma and its X63-m-IL-2 derivative.

The effect of irradiation on the expression of the CD80 molecule in tumour cells has also been tested previously (Morel et al., 1998; Seo et al., 1999). The CD80 molecule has been found to be neoexpressed after irradiation, and this neoexpression was reported to be dependent on the tissue of origin of the tumour cell lines (Morel et al., 1998). Morel et al. (1998) detected the neoexpression of the CD80 molecule after irradiation in P815 mastocytoma, as well as in mhAT2 and mhAT3F hepatomas, whereas no CD80 neoexpression could be observed in M116 leiomyoma and MCA-101 fibrosarcoma. The neoexpression of CD80 molecules has also been reported in liver cells after HCV infection (Mochizuki et al., 1997) or after cultivation of B16 melanoma in GM-CSF, IL-2, IFNα or γ (Hersey et al., 1994). The data concerning the neoexpression of the CD80 molecule in some irradiated tumours (Morel et al., 1998; Seo et al., 1999) are in accordance with our findings.

Taken collectivelly, our results suggest that upregulated expression of the CD80 molecule, together with higher IL-2 production, are responsible for the higher therapeutic efficacy of the irradiated X63-m-IL-2 tumour vaccines in the experimental plasmacytoma system examined.

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