

## Short Communication

# Intratumoral IL-2 Gene Transfer Improves the Therapeutic Efficacy of IL-12 but Not IL-18

(tumour immunotherapy / IL-2 / IL-12 / IL-18)

V. SOBOTA, J. BUBENÍK, J. ŠÍMOVÁ, T. JANDLOVÁ

Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Prague, Czech Republic

**Abstract.** We have compared the therapeutic activity of IL-12 and IL-18 in mice carrying IL-2 gene-transduced syngeneic sarcoma Mc12. The IL-2 gene-transduced sarcoma has previously been utilized as an irradiated, genetically modified tumour vaccine. Murine recombinant IL-12 was capable of suppressing growth of the IL-2 gene-modified sarcoma Mc12 in syngeneic mice more efficiently than growth of the parental Mc12 sarcoma. In contrast, murine recombinant IL-18 could neither inhibit growth of the parental Mc12 sarcoma, nor suppress growth of its IL-2 gene-modified transfectant. These results suggest that although both of these cytokines are functionally related and participate in the induction of IFN  $\gamma$  production as well as in cell-mediated immune cytotoxicity, in the murine sarcoma system only IL-12 is therapeutically active and exerts its therapeutic effect in concert with the IL-2 gene. Thus, intratumoral IL-2 gene transfer improves the therapeutic efficacy of IL-12; administration of recombinant IL-12 should therefore be considered as adjuvant in IL-2 gene therapy with irradiated, genetically modified tumour vaccines.

The gene coding for interleukin-2 (IL-2) was previously inserted into murine sarcoma Mc12 and tumorigenicity of a variety of cell clones with different expression of the inserted gene was assessed. It has been found that most of the genetically manipulated and IL-2-producing Mc12 cell clones were less tumorigenic than the parental Mc12 cell population; the tumorigenicity of the clones declined with increasing production of IL-2 (Rössner et al., 1997). Preimmunization of mice with the Mc12-IL-2<sup>+</sup> vaccine induced regression of a subsequent parental Mc12 challenge inoculum in a proportion of Mc12 tumour-in-

oculated mice after temporary growth of the tumour. Areas of necrosis and extensive infiltration with Mac1<sup>+</sup> and CD4<sup>+</sup> leukocytes have been observed in the regressing sarcomas (Bubeník et al., 1997). Subsequently, irradiated Mc12-IL-2<sup>+</sup> cells were utilized as vaccines for gene therapy of surgical minimal residual Mc12 sarcoma disease and were found to be efficient (Vlk et al., 1998). In order to optimize the therapeutic effects of the Mc12-IL-2<sup>+</sup> vaccines, the therapeutic efficacy of two other interleukins with well-established antitumour activity, IL12 (for a review, see Trinchieri, 1998) and IL-18 (for a review, see Lebel-Binay et al., 2000), both participating in the induction of interferon  $\gamma$  (IFN  $\gamma$ ) production as well as in cell-mediated immune cytotoxicity, were investigated in this paper. Experiments were designed to assess whether intratumoral IL-2 gene transfer could improve the therapeutic efficacy of IL-12 and IL-18.

## Material and Methods

### *Mice*

C57 BL/10 female mice, 2 – 4 months old, were obtained from Anlab Co., Prague, Czech Republic.

### *Tumour cells*

The Mc12 cell line derived from a poorly immunogenic (Bubeník et al., 1978; Vondrys et al., 1997) 3-MC-induced fibrosarcoma of C57 BL/10 origin was transfected with murine IL-2 cDNA as described earlier (Bubeník et al., 1997; Rössner et al., 1997). The clones of the Mc12 cells transfected with IL-2 cDNA were previously characterized with regard to the expression of the inserted genes and tumorigenicity (Rössner et al., 1997). From these clones, a clone designated as Mc12-IL-2-I-2, producing 300 i.u. IL-2/10<sup>6</sup> cells/24 h, was selected for the present experiments and characterized with regard to its tumorigenicity (Table 1). As can be seen in Table 1, the parental Mc12 cell population can grow in 100% syngeneic mice after s.c. inoculation of  $1 \times 10^5$  cells (log LD<sub>50</sub> = 3.5), whereas the tumorigenicity of the IL-2-producing clone Mc12-IL-2-I-2 is substantially lower; these cells grow in 100% mice after s.c. inoculation of  $5 \times 10^6$  cells (Tables 2, 3).

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Corresponding author: Jan Bubeník, Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Flemingovo nám. 2, 166 37 Praha 6, Czech Republic. Fax: 420 (2) 24310955; e-mail: bubenik@img.cas.cz.

Abbreviations: IL – interleukin, IFN – interferon, GM-CSF – granulocyte-monocyte colony-stimulating factor.

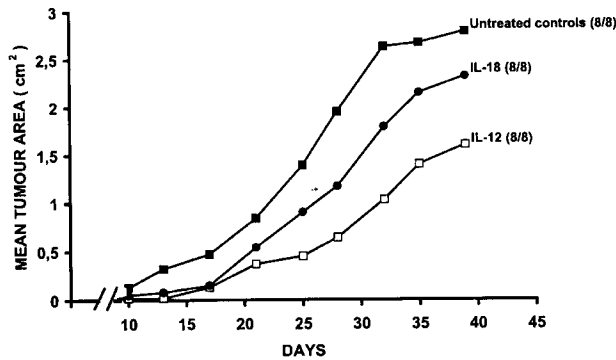


Fig. 1. Peritumoral administration of IL-12 inhibits growth of Mc12 sarcoma in mice. Untreated controls  $\times$  IL-12 –  $P < 0.05$ ; untreated controls  $\times$  IL-18 –  $P > 0.05$ . Number of mice with tumours / Total No. of mice.

### Interleukins

Murine recombinant IL-12 and murine recombinant IL-18 were purchased from R & D Systems, Minneapolis, MN, and utilized for peritumoral administration in a dose of 0.3  $\mu\text{g}/\text{mouse}$ , twice a day, for 5 days (on days 3 – 7 after challenge). For statistical analyses of the interleukin growth-inhibitory effects,  $\chi^2$  test and All Pair-wise Multiple Comparison Procedures, Student-Newman-Keuls Method, were utilized.

### Results

As can be seen in Fig. 1 and Table 2, murine recombinant IL-12 was capable of suppressing growth of both, the parental Mc12 sarcoma and its IL-2 transfectant Mc12-IL-2-I-2 in syngeneic mice. However, the Mc12-IL-2-I-2 cells were substantially more sensitive to the anti-cancer effect of IL-12 than their parental counterparts. In mice inoculated with the parental Mc12 cells a significant tumour-inhibitory effect of peritumoral IL-12 administration could only be detected with small ( $1 \times 10^5$ ) but not large ( $5 \times 10^6$ ) tumour inocula. The effect was expressed as inhibition of tumour growth (Fig. 1); regressions of the challenge inocula were never observed (Table 2). In contrast, in mice inoculated with the Mc12-IL-2<sup>+</sup> transfectant, the tumour-inhibitory effect could be detected with both, small and large tumour inocula; the effect was expressed not only as inhibition of tumour growth (data not shown), but also as regressions of

Table 1. Insertion of the IL-2 gene down-regulates tumorigenicity of Mc12 sarcoma cells.

Cell line	Cell dose				
	$10^4$	$10^5$	$10^6$	$10^7$	$10^8$
Mc12	6/7 <sup>a</sup>	7/7	7/7	5/5	5/5
Mc12-IL-2-I-2	0/7	2/7	4/7	7/7	5/5

<sup>a</sup>No. of mice with tumour/Total No. of mice

Table 2. Interleukin 12 suppresses growth of IL-2 gene-modified Mc12 cells more efficiently than growth of the parental Mc12 sarcoma.

Group of mice	Challenge inoculum <sup>a</sup>			
	Mc12		Mc12-IL-2-I-2	
	$1 \times 10^5$	$5 \times 10^6$	$1 \times 10^5$	$5 \times 10^6$
Treated with IL-12	8/8 <sup>b,c</sup>	7/7	0/7	7/13 <sup>d</sup>
Untreated controls	8/8	7/7	2/7	18/18

<sup>a</sup>No. of cells/mouse injected s. c., 5 days prior to therapy

<sup>b</sup>No. of mice with tumour/Total No. of mice

<sup>c</sup>A significant ( $P < 0.05$ ) inhibition of tumour growth not expressed as tumour regression was observed in IL-12 treated mice (see Fig. 1).

<sup>d</sup> $P < 0.01$  as compared to untreated controls

Table 3. Interleukin 18 could inhibit neither parental Mc12 sarcoma nor its IL-2 transfectant.

Group of mice <sup>a</sup>	Challenge inoculum <sup>b</sup>			
	Mc12		Mc12-IL-2-I-2	
	$1 \times 10^5$	$5 \times 10^6$	$1 \times 10^5$	$5 \times 10^6$
Treated with IL-18	8/8 <sup>c</sup>	7/7	2/7	8/8
Untreated controls	8/8	7/7	1/7	8/8

<sup>a</sup>Neither IFN ( $1 \mu\text{g}$  injected twice a day on days 3 – 5 or 8 – 10 after challenge), nor GM-CSF (20 ng injected twice a day on days 3 – 5 or 8 – 10 after challenge) could inhibit Mc12 and Mc12-IL-2-I-2 tumour growth. Both cytokines were also unable to potentiate the effect of IL-12 (data not shown).

<sup>b</sup>No. of cells/mouse injected s.c., 5 days prior to therapy

<sup>c</sup>No. of mice with tumour/Total No. of mice

growing tumours in a proportion of tumour-bearing mice (Table 2). These experiments were three times repeated with similar results and a typical result is shown in Table 2.

Using the same experimental protocol and the same doses of the interleukin, the experiments were also performed with IL-18. In contrast to IL-12, murine recombinant IL-18 could neither inhibit growth of the parental Mc12 sarcoma, nor suppress growth of the Mc12-IL-2-I-2 cells (Table 3). Similarly, neither mouse recombinant IFN  $\gamma$  (Sigma, St. Louis, MO) nor mouse recombinant GM-CSF (Sigma, St. Louis, MO) could inhibit Mc12 or Mc12-IL-2-I-2 tumour growth in syngeneic mice (Table 3, data not shown). Also these experiments were repeated with similar results.

### Discussion

Therapy of cancer with antitumour cytokines administered as recombinant products of cytokine genes (for a review, see Kedar and Klein, 1992; Verbik and Joshi, 1995) or as cytokine gene-modified and irradiated tumour vaccines (for a review, see Bubenik, 1996) remains to be optimized. Exciting results obtained in some