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Communication

Inhibition of tumor growth by interleukin 10 gene transfer in B16(F10) melanoma cells°

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Interleukin 10 (IL-10) is a potent immunosuppressive cytokine with an antitumor activity. The effect of IL-10 on tumor growth was tested in murine melanoma cells manipulated by gene transfer to secrete IL-10. In mice bearing B16(F10) tumors expressing IL-10 tumor growth was decreased depending on the amount of secreted IL-10.

Interleukin 10 (IL-10) is a multifunctional cytokine produced by different cell types including activated T cells [1], macrophages, monocytes [2], and keratinocytes [3]. IL-10 has been shown to inhibit cell mediated immunity and appears to be a natural anti-inflammatory agent [4, 5]. Increased expression of IL-10 was observed in tumor tissues and serum from patients with various types of cancer, including gliomas [6], carcinomas [7] and melanomas [8]. This, together with immunosuppressive properties of IL-10, suggests that IL-10 production in tumors suppresses antitumoral immune responses enabling, in fact, tumor development. Further investigations unexpectedly revealed antitumor activity of IL-10. Several studies demonstrated that IL-10 is a potent inhibitor of tumor growth and metastasis in animal models [9–15]. Therapeutic effect of IL-10 was described for several tumor types, including murine [13] and human melanoma [13, 16], murine mammary adenocarcinoma [14], murine sarcomas [15] and murine lung adenocarcinoma [13]. The antitumor effect of IL-10 appears to be the result of its activity inhibiting angiogenesis within tumor [10, 16].

The aim of our study was to examine the effect of IL-10 on B16(F10) murine melanoma tumor growth upon transfer of IL-10 gene into

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tumor cells. We cloned the IL-10 gene into pBCMGSNeo plasmid and transfected B16(F10) cells with this vector. Two clones producing different amounts ("high" and "low") of IL-10 were used in an *in vivo* experiment.

MATERIALS AND METHODS

Construction of pBCMGSNeo/mIL-10 plasmid. We used pBCMGSNeo expression vector (kindly provided from Dr. Karasuyama from Basel Institute of Immunology) [17]. pCDSRalpha plasmid encoding murine IL-10 cDNA was purchased from ATCC (No. 68027). *Bam*HI-*Bam*HI fragment from pCDSRalpha/mIL-10 construct containing mIL-10 cDNA [18] was cloned into the pBluescriptSK(+) to generate convenient restriction sites at the insert's ends. The *Xho*I-*Not*I insert from pBluescriptSK(+)/mIL-10 was then cloned into pBCMGSNeo expression vector. pBCMGSNeo/mIL-10 vector contains the IL-10 coding sequence under the transcriptional control of cytomegalovirus promoter with a rabbit β-globin intron and neomycin resistance gene.

Transfection of B16(F10) cells. B16(F10) cells (murine melanoma line) were transfected with pBCMGSNeo/mIL-10 expressing vector or pBCMGSNeo empty vector using Lipofectin (Gibco-BRL). After selection with G-418 (1 mg/ml) (Sigma-Aldrich), neomycin-resistant colonies were cloned and expanded. All B16(F10) cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (Gibco-BRL). Cultures were kept at 37°C in 5% CO_2 incubator.

IL-10 ELISA. IL-10 production was assessed in cell-conditioned medium by ELISA kit for murine IL-10 (Cytimmune Sciences Inc, Maryland, U.S.A.). Two IL-10 producing clones were used for this study. The 48-h IL-10 secretion was assayed after plating 5×10^5 cells in 25 cm^2 bottles containing 4 ml of medium. Clones producing different amounts of IL-10 were used in *in vivo* experiments.

Analysis of tumor growth. 6-8-week old C57Bl/6 mice were from our own animal facility. Mice had their left dorsal side shaved and then were inoculated subcutaneously with IL-10 transfected B16(F10) clones (2.5×10^5 cells per mouse) suspended in 100 μl PBS (phosphate buffered saline without Mg^{2+}, Ca^{2+}). Size of tumors was calculated from the following formula: volume = $(\text{width})^2 \times \text{length} \times 0.52$ [19].

RESULTS AND DISCUSSION

Table 1 shows the amounts of IL-10 secreted by B16(F10) cell lines transfected with the pBCMGSNeo/mIL-10 expressing vector. Clones #1 and #2 were found to produce different levels of IL-10. Clone #2 produced relatively high amounts of IL-10 (1×10^4 pg/ml) while clone #1 produced lower levels of IL-10 (6.1×10^3 pg/ml). Parental B16(F10) cells and control B16(F10)/pBCMGSNeo cells did not secrete a detectable amount of IL-10.

To study *in vivo* effect of IL-10 local secretion on tumor growth, IL-10 expressing B16(F10) clones and control cells were injected into the flank of syngenic mice. As shown in Fig. 1, mice injected with parental B16(F10) cells or cells transfected with empty vector developed rapidly growing tumors. Mice injected with clones #1 and #2 secreting IL-10 developed tumors much slower. Therapeutic effect was dependent on the amount of IL-10 produced by tumor cells. Tumors derived from the cell clone producing higher amount of IL-10 (#2) have slower growth kinetics compared to the cell clone producing lower level of the cytokine (#1) (Fig. 1).

Our experiments confirmed earlier reports of antitumor properties of IL-10 in B16 tumors [10, 11]. In B16(F10) tumors negative correlation was found between the amount of IL-10 secreted by IL-10-gene transfected cells and both tumor growth and metastatic spread [10]. However, little is known about the mechanism by which locally secreted IL-10 inhibits

Table 1. Interleukin 10 secretion by B16(F10) clones transfected with pBCMGSNeo/mIL-10.

Several single clones of B16(F10) cells transfected with pBCMGSNeo empty plasmid or pBCMGSNeo/mIL-10 were isolated and the level of IL-10 in cell-conditioned medium was quantified by ELISA.

Cell line	IL-10 secretion (pg/ml)
B16(F10)/pBCMGSNeo/mIL-10 #1	6.1×10^3
B16(F10)/pBCMGSNeo/mIL-10 #2	1×10^4
B16(F10)/pBCMGSNeo	Not detected
B16(F10)	Not detected

melanoma growth. It has been shown that IL-10 acts as inhibitor of angiogenesis within tumors by inactivating some macrophages' functions responsible for neovascularization [5, 16]. It is also speculated that IL-10 expression by tumors leads to enhanced NK lysis of tumor cells [11, 13]. Kundu *et al.* [20] have shown that IL-10 indirectly induces an increase of nitric oxide level at the tumor site which is associated with tumor inhibition. On the other hand, there are reports which emphasize immunosuppressive and anti-inflammatory properties of IL-10 and point to IL-10 as a growth factor for tumor cells *in vitro* [6, 21]. Therefore more profound understanding of this cytokine mechanism of action is necessary. This could possibly lead to a powerful method of therapeutic intervention in a wide variety of cancers.

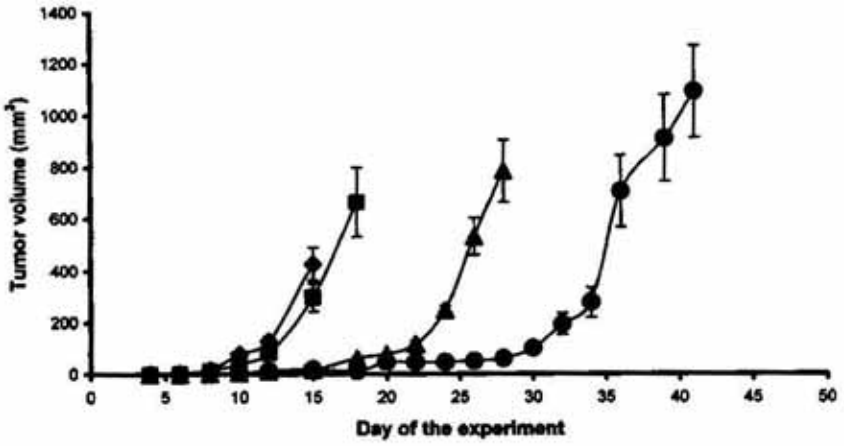

Figure 1. Tumor growth in mice inoculated with B16(F10) cells expressing different amounts of interleukin 10.

C57Bl/6 mice were inoculated subcutaneously with B16(F10) cells' suspension (2.5×10^5 cells/animal) expressing different amounts of IL-10 (#1, #2) or control B16(F10) (◆) or B16(F10)/ pBCMGSNeo control cells (■). B16-(F10)/pBCMGSNeo/mIL-10 cells #1 (▲) produce 6.1×10^3 pg/ml; B16(F10)/pBCMGSNeo/mIL-10 cells #2 (●) produce 1×10^4 pg/ml. Experimental groups numbered 5 animals and each point represents the mean tumor volume of all 5 mice. These data are representative for two independent experiments.

Described here reduced tumorigenicity of B16(F10) melanoma cells secreting IL-10 indicates that tumor gene therapy with IL-10 is possible and might be efficient, all the more that systemic administration of IL-10 induces inhibition of tumor growth and metastasis in animal models [13, 15] and causes no adverse or toxic effect in humans [22].

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