Modulation of Transforming Growth Factor- β Secretion from Malignant Glioma Cells by Interleukin-1 β

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Abstract

Malignant glioma cells secrete transforming growth factor- β (TGF- β) which has potent immunosuppressive properties. We investigated the effect of interleukin-1 β (IL-1 β) on TGF- β secretion from malignant glioma cells *in vitro*. T98G glioblastoma cells were treated with various doses of IL-1 β and the TGF- β activity in the supernatant was determined using a specific bioassay. Six other human malignant glioma cell lines were also treated with 1000 U/ml of IL-1 β , and the TGF- β activity in the supernatants was determined. The effect of IL-1 β on the growth of tumor cells was also assessed by a bioassay using crystal violet which reflects the actual cell number in the plate wells. IL-1 β treatment resulted in inhibition of TGF- β secretion in two malignant glioma cell lines. TGF- β secretion from T98G cells was suppressed by IL-1 β in a dose-related manner. However, IL-1 β treatment resulted in an obvious increase (>20%) of TGF- β secretion in two tumor lines, and a slight increase (<20%) in three tumor lines. IL-1 β did not affect the growth of four malignant glioma cell lines, and only slightly affected the growth of the other three cell lines. IL-1 β modulates TGF- β secretion from malignant glioma cells, but not in a consistent way.

Key words: malignant glioma, transforming growth factor- β , interleukin-1 β

Introduction

Malignant glioma cells secrete or express several cytokines, including interleukin-1 (IL-1),^{12,16,21)} IL-6,^{4,30,44,46)} IL-8,^{25,43,46)} granulocyte-macrophage colony-stimulating factor,¹⁴⁾ fibroblast growth factor,³⁹⁾ platelet-derived growth factor,²⁹⁾ insulin-like growth factor,³⁴⁾ transforming growth factor- α (TGF- α),²³⁾ and TGF- β .^{2,6,28,35,45)} These cytokines apparently interact and affect the local immune reaction, as well as regulate the growth of tumor cells. TGF- β has immunosuppressive properties, including inhibition of T cell proliferation,¹⁹⁾ IL-2 receptor induction,¹⁹⁾ cytokine production,^{9,10,27)} natural killer cell activity,³³⁾ cytotoxic T lymphocyte development,³²⁾ lymphokine-activated killer cell generation,¹⁰⁾ and production of tumor-infiltrating lymphocytes.²⁰⁾ Most types of cells secrete TGF- β in a latent form,³⁷⁾ but TGF- β exerts its biological function after conver-

sion to an active form.³⁸⁾ Our previous studies showed that malignant glioma cells secrete both TGF- β 1 and TGF- β 2, and could convert TGF- β from the latent to active form,^{28,35)} and that TGF- β secreted by glioblastoma cells inhibited tumor necrosis factor- α and β secretion by lymphokine-activated killer cells stimulated with tumor cells.²⁷⁾ These observations suggest that TGF- β secretion from malignant glioma cells may have some role in the immunosuppressive state of patients, in accordance with previous reports.^{20,24)} Other cytokines may regulate TGF- β secretion from malignant glioma cells, but this area has been little investigated.

To develop a successful immunological approach to the treatment of patients with malignant glioma, the mechanism or substance that regulates TGF- β secretion should be identified. In this study, we investigated the role of IL-1 β in TGF- β secretion from malignant glioma cells, and demonstrate the first evidence that IL-1 β modulates TGF- β secretion from malignant glioma cells.

Materials and Methods

I. Tumor cell culture

Human malignant glioma cell lines (T98G, A172, U251, U87, YMG2, YMG3, YMG4) were maintained in RPMI1640 medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 μ g/ml kanamycin, and 0.05 mM 2-mercaptoethanol. T98G and A172 cell lines were obtained from the Japanese Cancer Research Resources Bank, Tokyo. U251 and U87 cell lines were donated by Dr. Masaki Kurimoto, Toyama Medical and Pharmaceutical University, Toyama. YMG2, YMG3, and YMG4 cell lines were established in our laboratory. YMG2 was derived from anaplastic astrocytoma, and YMG3 and YMG4 from glioblastoma. Immunohistochemical analysis showed the YMG2, YMG3, and YMG4 tumor lines were positive for glial fibrillary acidic protein and S-100 protein. T98G, A172, YMG2, YMG3, and YMG4 cells secreted both TGF- β 1 and TGF- β 2, and could convert TGF- β from the latent to active form.^{28,35}

II. IL-1 β treatment

Preliminary studies indicated that IL-1 β treatment caused a marked reduction in TGF- β secretion from T98G cells among various malignant glioma cell lines. Therefore, the effect of various doses of IL-1 β added to culture medium on the inhibition of TGF- β secretion from T98G cells was examined. Subconfluent T98G cells cultured in 6-well plates (Nunc, Roskilde, Denmark) were treated with different concentrations of recombinant human IL-1 β (1, 10, 100, 1000 U/ml) (Genzyme, Cambridge, Mass., U.S.A.) for 16 hours. After washing three times with phosphate buffered saline, T98G cells were cultured in 1.5 ml of serum-free medium with 1 mg/ml of bovine serum albumin for 24 hours. Supernatants were harvested, filtered (0.22 μ m), and stored -20°C until use.

The other six human glioma cell lines cultured in 6-well plates were treated with 1000 U/ml of IL-1 β for 16 hours. After washing three times with phosphate buffered saline, tumor cells were incubated in serum-free RPMI1640 medium with 1 mg/ml of bovine serum albumin for 24 hours. Supernatants were harvested, filtered, and stored at -20°C until use.

III. TGF- β bioassay

The levels of TGF- β in the culture supernatants were determined by the bioassay using Mv1Lu mink lung epithelial cells described elsewhere.²⁸ Briefly, the supernatants were untreated or acid-treated (1.5 μ l 6 N HCl/200 μ l supernatant) for 15 minutes at

room temperature. The acid-treated samples were then neutralized with 1.5 N NaOH to a final pH of 7–7.4. To determine whether the growth inhibition of Mv1Lu cells is due to TGF- β , the untreated and acid-treated supernatants were pretreated with both anti-TGF- β 1 and anti-TGF- β 2 antibodies (20 μ g/ml) (R&D Systems, Minneapolis, Minn., U.S.A.) for 1 hour at 37°C . Fifty-microliter aliquots of the supernatants were plated in 96-well plates in duplicate and serial two-fold dilutions made in the plates. Fifty microliter aliquots of Mv1Lu cells (2000 cells) were added to each well. After 72 hours at 37°C in 5% CO_2 , wells were decanted and cells stained with 0.5% crystal violet for 5 minutes. After washing, the dye was eluted and the absorbance at 570 nm was determined. Human recombinant TGF- β 2 (Austral Biologicals, San Ramon, Cal., U.S.A.) was used as an internal standard. By determining the dilution of the test samples at which 50% inhibition occurs and extrapolating from the known recombinant TGF- β 2 dilution curve, the concentration of TGF- β , including TGF- β 1 and TGF- β 2, in the test samples was calculated.

Previous studies indicated that at least 10 μ g/ml of anti-TGF- β 1 or anti-TGF- β 2 antibody completely neutralized 2.5 ng/ml of natural human TGF- β 1 or recombinant human TGF- β 2, respectively, and that anti-TGF- β 1 antibody did not neutralize TGF- β 2 and *vice versa*.²⁸ The coefficient of variation of the bioassay was less than 20% at each concentration of recombinant TGF- β 2.

IV. Effect of IL-1 β on the growth of malignant glioma cells

The effect of IL-1 β on the growth of T98G cells was examined using a modified bioassay described elsewhere,²⁶ which reflected the actual cell number in well plates. Briefly, 100 μ l aliquots of IL-1 β (2000 U/ml) were plated in 96-well plates in quadruplicate and serial two-fold dilutions made in the plate. Fifty microliter aliquots of T98G cells (2000 cells) were added to each well. After incubating 72 hours at 37°C in 5% CO_2 , wells were decanted and cells stained with 0.5% crystal violet in 20% methanol for 5 minutes. After washing in water, the dye was eluted with 50% ethanol, and the absorbance at 570 nm was determined by a Titertek Multiskan (Flow Laboratories Inc., Helsinki, Finland).

Six-well plates, in which the other six malignant glioma cells had been treated with 1000 U/ml of IL-1 β and the supernatants harvested, were decanted. The cells in the plates were stained with crystal violet and the dye was eluted with 2 ml of 50% ethanol. 150 μ l aliquots of the eluted solution in each well was

Fig. 1 Effect of IL-1 β pretreatment on TGF- β secretion from T98G glioblastoma cells. T98G cells were pretreated with IL-1 β for 16 hours, and the supernatants were harvested after culturing for 24 hours. The amount of TGF- β in the supernatants includes both TGF- β 1 and TGF- β 2. The figure represents two experiments.

transferred to 96-well plates and the absorbance was determined.

Results

I. Modulation of TGF- β secretion by IL-1 β

Pretreatment of T98G cells with IL-1 β resulted in the inhibition of TGF- β secretion from T98G cells in a dose-related manner (Fig. 1). TGF- β secretion from T98G cells treated with IL-1 β (1000 U/ml) was suppressed by a maximum of 66%.

The response to IL-1 β treatment in the other six tumor lines showed different patterns. TGF- β secre-

Fig. 2 Effect of IL-1 β on the growth of T98G glioblastoma cells. Data are expressed as mean $\pm$ SD, compared with control cells.

tion decreased in U87 cells by 21% (Table 1). In contrast, A172 and YMG2 cells demonstrated an obvious increase of TGF- β secretion, showing 58% and 23% increases, respectively. U251, YMG3, and YMG4 cells showed slight increases of TGF- β secretion after IL-1 β treatment, but by less than 20%. TGF- β secretion after IL-1 β treatment in terms of cell density decreased in two tumor lines (T98G, U87), increased slightly in two lines (U251, YMG3), and increased obviously in three lines (A172, YMG2, YMG4).

IL-1 β did not affect the growth of Mv1Lu cells used in the bioassay. The TGF- β activity in test samples was completely neutralized with the combination of anti-TGF- β 1 and anti-TGF- β 2 antibodies.

Table 1 Effect of IL-1 β treatment on TGF- β secretion by malignant glioma cells

Tumor line	TFG- β content (ng/ml) in supernatants treated with				Change in total TGF- β after IL-1 β treatment (%)
	Medium		IL-1 β (1000 U/ml)		
	Active form	Total*	Active form	Total	
A172	UD	19.4	2.9 (2.8)	30.7 (30.1)	+58.2 (+55.2)
U251	4.3	22.4	4.5 (4.4)	26.8 (26.2)	+19.6 (+17.0)
U87	0.9	5.7	UD	4.5 (4.9)	-21.1 (-14.0)
YMG2	2.1	28.2	2.1 (2.1)	34.8 (34.4)	+23.4 (+22.0)
YMG3	UD	14.2	1.5 (1.6)	15.7 (16.4)	+10.6 (+15.5)
YMG4	UD	2.9	UD	3.3 (3.8)	+13.8 (+31.0)

Values in parentheses show TGF- β contents in the supernatant in terms of cell density in 6-well plates. *Total TGF- β content derived from acid-treated supernatants. UD: undetectable.

Table 2 Effect of IL-1 β treatment on the growth of malignant glioma cells

Malignant glioma cells treated with IL-1 β (1000 U/ml)					
A172	U251	U87	YMG2	YMG3	YMG4
101.8	102.1	90.8	101.1	95.6	85.8

Values are percentages of control cell number obtained by dividing optical density in IL-1 β -treated wells by that in control wells.

II. Effect of IL-1 β on the growth of malignant glioma cells

The growth of T98G cells was not affected by IL-1 β during short term culture (Fig. 2). IL-1 β inhibited slightly the growth of three tumor lines (U87, YMG3, YMG4), but the growth of the other tumor lines (A172, U251, YMG2) was not affected by IL-1 β (Table 2).

Discussion

The present study provides the first evidence that IL-1 β modulates TGF- β secretion from malignant glioma cells. IL-1 β treatment inhibits TGF- β secretion in some malignant glioma cells, but enhances TGF- β secretion in other tumor lines. Why IL-1 β treatment of malignant glioma cells had a different effect on TGF- β secretion from various tumor cell lines is unclear. In breast cancer cells, IL-1 β treatment increases secretion of TGF- β but this IL-1 β -induced TGF- β secretion is blocked by estradiol.⁵⁾ In normal rat pulmonary artery endothelial cells, IL-1 β treatment increases secretion of TGF- β , showing synergistic enhancement with tumor necrosis factor- α treatment, and causes expression of TGF- β messenger ribonucleic acid (mRNA).³¹⁾ In addition, IL-7 decreases TGF- β secretion from macrophages⁸⁾ and fibrosarcoma.⁷⁾ These observations suggest that other cytokines or factors than IL-1 β may be also related to the modulation of TGF- β secretion from malignant glioma cells.

Malignant glioma cells constitutively express IL-1 α or IL-1 β mRNA^{16,22,44)} and secrete IL-1 α or IL-1 β ,^{12,16,21)} and some glioma cells express the IL-1 receptor.⁴¹⁾ Furthermore, IL-1 β stimulates the secretion of IL-6,^{4,30,44,46)} IL-8,^{25,43,46)} and granulocyte-macrophage colony-stimulating factor¹⁴⁾ from malignant glioma cells. Together with the present results, these facts suggest that IL-1 β modulates the secretion of several cytokines from malignant glioma cells. In malignant glioma tissues, the cytokines tumor necrosis factor- α and IL-6 were most prominent in infiltrating macro-

phages in those regions that showed little immunoreactivity for TGF- β . In contrast, TGF- β immunoreactivity is high in tissue with little tumor necrosis factor- α -positive macrophage infiltration.³⁶⁾ These observations indicate a complex interaction between tumor cells and anti-tumor immunity.

TGF- β has several potent immunosuppressive properties,^{11,15,17,18,32)} and may limit tumor regression. Most cells secrete TGF- β as the latent form,³⁷⁾ but TGF- β only shows biological activity after conversion to the active form.³⁸⁾ Most normal cells cannot activate TGF- β , but some tumor cells can do so.⁴⁰⁾ Our previous studies suggested that most malignant glioma cells had the capacity to convert TGF- β from the latent to active form.^{28,35)} Further evidence for the down-regulation of antitumor responses is provided by the observation that a highly immunogenic murine tumor transfected with TGF- β complementary deoxyribonucleic acid escaped immune surveillance.⁴²⁾ Therefore, the acquisition of the capacity for TGF- β activation seems to be important for tumor cells to escape the immunosurveillance system and to continue to grow. Recent observations have shown that an active form of TGF- β is produced by cocultures of vascular endothelial cells and pericytes,¹⁾ vascular endothelial cells have tissue plasminogen activator activity,¹³⁾ and latent TGF- β is converted to the active form by plasmin.³⁾ Therefore, if tumor cells secrete abundant latent TGF- β , conversion to the active form may occur around blood vessels in tumor tissues. These observations agree with recent reports that TGF- β is relevant to the immunosuppressive states of the patients with malignant gliomas.^{20,24)}

Methods to downregulate TGF- β secretion from malignant glioma cells may become one of the treatment modalities for patients with malignant gliomas. Currently, the therapeutic abrogation of TGF- β activity is the focus of study in cancer patients.⁷⁾ However, IL-1 β alone may not be suitable for this purpose because the present study showed that IL-1 β upregulated the TGF- β secretion from some malignant glioma cells. Therefore, further study to elucidate the mechanism of the regulation of TGF- β secretion from malignant glioma cells is needed.

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