Laboratory Investigation

Down-regulation of transforming growth factor-β and interleukin-10 secretion from malignant glioma cells by cytokines and anticancer drugs

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Summary

The effect of treatment with interleukin-1β (IL-1β), interferon-γ (IFN-γ), vincristine, and etoposide was evaluated on the secretion of transforming growth factor-β (TGF-β) and IL-10 and the expression of major histocompatibility complex (MHC) class I, intercellular adhesion molecule-1 (ICAM-1), and CD80 molecules by malignant glioma cells. Five malignant glioma cell lines were treated with IL-1β, IFN-γ, and/or anticancer agents (vincristine and etoposide). Combined treatment with IL-1β and IFN-γ caused greater inhibition of TGF-β secretion compared to treatment with IFN-γ alone and almost the same levels of inhibition as treatment with vincristine and etoposide. The greatest inhibition of TGF-β secretion was achieved by treatment with all agents. Low levels of IL-10 secretion were determined in two out of five malignant glioma cell lines. This IL-10 secretion was inhibited by treatment with IL-1β, IFN-γ, vincristine, and/or etoposide. Treatment with both cytokines and anticancer agents increased the expression of MHC class I and ICAM-1 in all tumor cell lines. The mean increase of expression of MHC class I was 50% and that of ICAM-1 was 12-fold. No tumor cell lines expressed CD80 molecules on the cell surface, and no treatment caused CD80 expression. These results suggest that TGF-β and IL-10 secretion by malignant glioma cells can be suppressed by treatment with a combination of IL-1β, IFN-γ, vincristine, and etoposide, and the treatment up-regulates MHC class I and ICAM-1 expression on tumor cells. These results have implications for immunotherapy and chemotherapy in patients with malignant tumors.

Introduction

Malignant glioma cells secrete or express several cytokines, of which transforming growth factor-β (TGF-β) and interleukin-10 (IL-10) have immunosuppressive properties. IL-10 has a variety of effects, including inhibition of monocyte major histocompatibility complex (MHC) class II-dependent antigen presentation, type 1 helper T cell cytokine production, and inhibition of T cell proliferation [1, 2]. TGF-β inhibits T and B cell proliferation [3, 4]. IL-2 receptor induction [4], cytokine production [5, 6], natural killer cell activity [7], cytotoxic T lymphocyte development [8], lymphokine activated killer cell generation [6], and production of tumor-infiltrating lymphocytes [9]. TGF-β secreted by malignant glioma cells is involved in the immunosuppressive state of patients [9, 10]. In vivo, administration of TGF-β inhibits T cell response to allogeneic tumor cells [11].

Most cells secrete TGF-β in a latent form [12], which only manifests its biological functions after
conversion to an active form [13]. Three distinct TGF-β polypeptides, TGF-β1, TGF-β2, and TGF-β3, have been found in humans [14–17]. Malignant glioma secrete TGF-β1 and/or TGF-β2 [18–21], and have the potential to activate latent TGF-β [18–20].

TGF-β secretion by tumor cells may be regulated by other cytokines, in the same way that TGF-β inhibits the secretion of other cytokines such as tumor necrosis factor-α, tumor necrosis factor-β, and IFN-γ [5, 6, 22]. However, a method to down-regulate the secretion of TGF-β and IL-10 by tumor cells has not yet been found. So far, only dexamethasone or epidermal growth factor has been shown to inhibit TGF-β secretion in some tumor cells [23]. The immunological approach to treating malignant tumors seems to require a method to down-regulate TGF-β and IL-10 secretion and up-regulate the expression of MHC class I, intercellular adhesion molecule-1 (ICAM-1), and CD80 molecules on tumor cells. T cell activation by antigen presenting cell is mediated both by binding of T cell receptor/CD3 complex and MHC class I molecules [24], and by binding of lymphocyte functional antigen-1 and ICAM-1 [25]. Recent studies indicate that the interaction between CD80 and CD28 is also required for T cell proliferation [26] and the generation of cytotoxic T lymphocytes [27]. Therefore, up-regulation of MHC class I, and cell-adhesion molecules like ICAM-1 is at least necessary to induce an effective cellular immune response against tumor cells.

This study investigated the effect of cytokines (IL-1β, IFN-γ) and anticancer drugs (vincristine, etoposide) treatment on the secretion of TGF-β and IL-10 and the expression of MHC class I, ICAM-1, and CD80 molecules by malignant glioma cells. We demonstrated that treatment with IL-1β, IFN-γ, vincristine, and etoposide inhibited the secretion of TGF-β and IL-10 by tumor cells and increased the expression of MHC class I and ICAM-1, but not the CD80 molecule.

Materials and methods

Tumor cell culture

Human malignant glioma cell lines (T98G, A172, KG-1-C, YMG2, YMG4) and Raji cells were maintained in RPMI1640 medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 μg/ml kanamycin, and 0.05 mM 2-mercaptoethanol. T98G, A172, KG-1-C, and Raji cells were obtained from the Japanese Cancer Research Resources Bank, Tokyo, Japan. YMG2 and YMG4 tumor cell lines were established in our laboratory. YMG2 cell line was derived from an anaplastic astrocytoma, and YMG4 from a glioblastoma. YMG2 and YMG4 tumor cell lines were positive for glial fibrillary acidic protein and S-100 protein by immunohistological analysis. T98G and A172 cells secreted both TGF-β1 and TGF-β2, and could convert TGF-β from the latent to active form [19, 20].

Cytokine and anticancer agent treatment

IL-1β, IFN-γ, vincristine, and etoposide were used for the pretreatment of tumor cells, since previous studies [28] showed that TGF-β secretion by T98G cells was inhibited by pretreatment with IL-1β, IFN-γ, vincristine, and etoposide among various cytokines (IL-1β, IFN-γ, tumor necrosis factor-α, tumor necrosis factor-β, IFN-β) and anticancer agents (vincristine, etoposide, ACNU [1-(4-amino-2-methyl-5-pyrimidinyl)methyl-3-(2-chloroethyl)-3-nitrosourea hydrochloride], carboplatin, mitomycin C, anthomycin D).

Five malignant glioma cell lines (T98G, A172, KG-1-C, YMG2, YMG4) were cultured in 6-well plates until subconfluent. The following were then added to separate wells: 1) culture medium with 10% fetal bovine serum, 2) recombinant human IL-1β (500 U/ml) (Genzyme, Cambridge, MA), 3) recombinant human IFN-γ (500 U/ml) (Toray Co., Ltd., Tokyo, Japan), 4) IL-1β (500 U/ml) and IFN-γ (500 U/ml), 5) vincristine (1 μg/ml) (Wako Pure Chem. Ind., Ltd., Osaka, Japan) and etoposide (50 μg/ml) (Wako Pure Chem. Ind., Ltd.), and 6)