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Laboratory Investigation

Dexamethasone mediated inhibition of local IL-2 immunotherapy is dose dependent in experimental brain tumors

Maciej S. Lesniak[1,2], Patrick Gabikian, Betty M. Tyler, Drew M. Pardoll[3] and Henry Brem[2,3]
[1]*Division of Neurosurgery, The University of Chicago Hospitals, Chicago, IL;* [2]*Department of Neurosurgery;* [3]*Department of Oncology, The Johns Hopkins School of Medicine, Baltimore, MD, USA*

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Summary

Local delivery of cytokines has been shown to have a potent anti-tumor activity against a wide range of malignant brain tumors. In this study, we examined the role of systemic immunosuppression using dexamethasone on the efficacy of local IL-2 immunotherapy in treating experimental murine CNS tumors.

An endothelial cell line secreting hIL-2 (NTC-121) was injected intracranially in C57BL/6 mice ($n = 10$/group) along with B16/F10 (wild type) melanoma cells. A separate set of animals also received daily injections of either 1 mg/k or 10 mg/kg of dexamethasone. Sixty percent of mice treated with IL-2 ($P < 0.001$ vs. control) vs. 55% ($P < 0.005$) of mice treated with IL-2 and 1 mg/kg of dexamethasone were long-term survivors (LTS) of >120 days. There was no difference in survival between control animals that received only wild type cells or animals that were treated with IL-2 and 10 mg/kg of dexamethasone. Histopathological examination of brains from animals sacrificed at different times showed an accumulation of CD8 + T-cells around the site of the injected tumor only in the IL-2 group and the group that received 1 mg/kg of dexamethasone.

These results suggest that while high doses of dexamethasone can completely inhibit the immune response observed with IL-2, lower and more likely therapeutic doses of dexamethasone do not inhibit local IL-2 immunotherapy.

Introduction

For patients with malignant gliomas, new therapeutic approaches have focused on treatment strategies that target tumor cells and spare normal cells. One such modality, immunotherapy, has shown promise in the spectrum of agents utilized against malignant brain tumors. Specifically, local delivery of interleukin-2 (IL-2), a potent stimulator of immune effector cells, has been shown to significantly prolong the survival of animals with experimental brain tumors [1–9].

To date, all of the experiments utilizing IL-2 have been performed in immunocompetent mice. However, almost all patients with malignant brain tumors require dexamethasone at some point in their treatment, and the effects of steroids on local response to IL-2 immunotherapy have not been examined in any of the previous studies. Dexamethasone, in particular, acts directly to inhibit the secretion of IL-2 and suppress the response of the T cells to its potent immunomodulatory function [10,11]. As a result, our objective in this study was to determine what effects, if any, systemic administration of

dexamethasone would have on local immunotherapy with IL-2.

Materials and methods

Cell lines and animals

B16/F10 melanoma cells were obtained from the National Cancer Institute-Division of Cancer Treatment and Diagnosis Tumor Repository (Frederick, MD). The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal calf serum and penicillin/streptomycin.

A rat endothelial cell line (NTC-121) was provided by Neurotech, S.A. The cells were cultured in a 50:50 medium containing Alpha Minimum Essential Medium (Life Technologies) and Ham's F10 (Life Technologies) supplemented with 0.25 ng/ml human bFGF (Life Technologies) and 10 μg/ml of rat tail type I collagen (B & D). The NTC-121 cells were transduced with the human IL-2 gene by using the MFG retroviral vector, as previously described [12]. The amount of IL-2 produced by the transformed cells was measured before each experiment by a standard ELISA technique (Endogen, Cambridge, MA).

Cultured monolayers were harvested with trypsin and resuspended in DMEM before injection. C57BL/6 female mice (6–12 weeks old) were obtained from Harlan.

Experimental intracranial model

Mice were anesthetized with an intraperitoneal injection of 0.1 ml of a stock solution containing ketamine hydrochloride 25 mg/ml, xylazine 2.5 mg/ml, and 14.25% ethyl alcohol diluted 1:3 in 0.9% NaCl. For stereotactic intracranial injections of tumor cells, the surgical site was shaved and prepared with 70% ethyl alcohol and Prepodyne solution. After a midline incision, a 1 mm right parietal burr hole centered 2 mm posterior to the coronal suture and 2 mm lateral to the sagittal suture was made. Animals were then placed in a stereotactic frame and cells were delivered by a 26 gauge needle to a depth of 3 mm over a period of 3 min. The total volume of injected cells was 5 μl. The needle was removed, the site was irrigated with sterile 0.9% NaCl, and the skin was sutured with 4.0 vicryl.

Intracranial cytokine studies

The toxicity and efficacy studies of NTC-121 cells have been previously published [9]. All animals ($n = 10$/group) were treated with stereotactic intracranial injections of 100 live non-irradiated, non-cytokine producing B16/F10 melanoma cells. Injection of these cells has been shown to produce a large tumor at the injection site that is uniformly fatal, with a median survival between 16 and 18 days [9,10]. Each of the animals then received 4×10^6 NTC-121 cells producing 250 ng/10^6 cells/24 h of IL-2. The results are based on three independent sets of experiments performed over the course of this study.

Dexamethasone treatment

Beginning on the day of tumor implantation, the animals were given daily intraperitoneal injections of dexamethasone (1 or 10 mg/kg) or PBS (control animals).

Histological evaluation

One set of animals ($n = 10$/group) was set aside for the purpose of histopathological examination. The animals were then euthanized on days 1, 4, 9, and 14. The brains were removed, the tissue was fixed in 10% formalin, blocked in paraffin, sectioned in coronal plane in 10 μm sections, and stained with hematoxylin and eosin (H&E). Immunohistochemistry using the peroxidase anti-peroxidase technique was also used with the following primary antibodies: anti-CD3, -CD4, -L26, or -CD8 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). A murine lymph node was used as a positive control.

Statistical analysis

For all efficacy studies, survival was the primary endpoint. All animals were monitored for any sign of neurotoxicity and autopsied, when possible, to confirm that death was due to intracranial tumor. Survival was plotted using a Kaplan–Meier survival analysis and statistical significance was

determined by the Kruskal–Wallis non-parametric analysis of variance followed by the non-parametric analog of the Newman–Keuls multiple comparison test [13].

Results

IL-2 successfully prolongs the survival of mice with experimental brain tumors

Sixty percent of mice treated with IL-2 ($p < 0.001$ vs. control) were long-term survivors (LTS) of >120 days. Control animals that received only wild type cells had a median survival of 18 days (range 15–20) (Figure 1).

Inhibition of local IL-2 immunotherapy is related to the dose of systemic dexamethasone

Of the mice receiving daily injections of 1 mg/kg of dexamethasone, 54% were LTS of >120 days ($p < 0.005$). In contrast, there was no difference in median survival or overall survival in mice treated with 10 mg/kg of dexamethasone or control animals (Figure 1).

Mice treated with xenogenic IL-2 show an accumulation of CD8 T cells

To better understand the anti-tumor effects of IL-2, we stained animal brains with different tissue markers. Whereas all animals treated with IL-2 reacted strongly with the T cells marker CD3, none of them showed any evidence of an L26 B cell response. Furthermore, when examined for CD4 or CD8 markers, the mice treated with IL-2 stained negatively for CD4 and positively for CD8 (Figure 2). Administration of dexamethasone at a dose of 1 mg/kg had no effect on the presence of the infiltrate whereas 10 mg/kg was associated with minimal to no infiltrate (Figure 2). These results showed no variation in the type of immune infiltrate over a course of 2 weeks.

Discussion

The development of novel immunotherapeutic approaches for the treatment of brain tumors has gained increasing attention during the past decade. Direct, local delivery of cytokines such as IL-2 has been shown to prolong the survival of animals with experimental brain tumors [1–3,5,9]. The success of immunotherapy, however, depends in part on a functional immune system capable of mounting and sustaining an anti-tumor response. In the case of patients with glioblastoma multiforme, the majority receive long-term treatment with steroids, and as such have an overall depressed immune system. The role of this paper has been to examine whether the success of IL-2 previously reported in the treatment of experimental brain tumors is affected by systemic administration of a potent steroid, such as dexamethasone.

Dexamethasone is one of the most powerful corticosteroids, first utilized in the management of patients with inflammatory disorders and transplant recipients. While its exact mechanism of action is unknown, it has been shown to inhibit leukocyte trafficking and induce apoptosis in human lymphocytes and monocytes [14–16]. Despite its widespread use in glioma patients, the immunosuppressive function of dexamethasone in brain tumors has been rarely mentioned in any studies. Neuwelt et al., for instance, demonstrated that peripheral lymphocytes isolated from glioma patients were less responsive to mitogens in the presence of dexamethasone [17]. Understanding the immunosuppressive mechanism of steroid medications in patients with brain tumors and the doses at which they exert these effects should prove helpful in designing more effective immune-system-based therapies.

In this study, we looked at two different concentrations of dexamethasone (1 and 10 mg/kg). The choice of these concentrations was based on the therapeutic range used in patients with gliomas and experimental dosage used in animal studies [18]. Our results show that the survival of mice treated with IL-2 in not affected by doses of decadron up to 1 mg/kg. Indeed, over 50% were LTS. In contrast, mice receiving 10 mg/kg of dexamethasone showed no survival benefit as compared to control animals. These findings suggest that local immunotherapy with IL-2 can be observed in the context of systemic immunosuppression with low doses of decadron. However, increasing the dose of dexamethasone directly inhibits the immune response to IL-2.

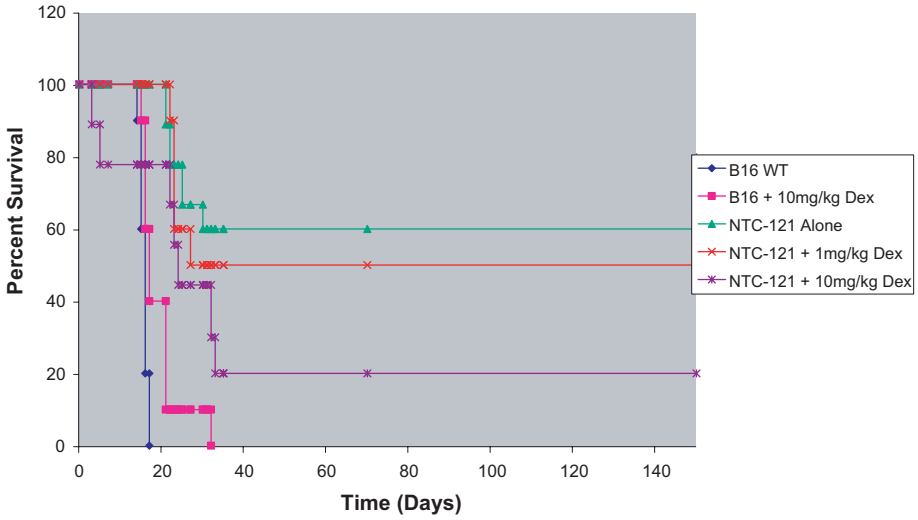

Figure 1. Survival of mice ($n = 10$/group) after intracranial delivery of NTC-121 cells and intracranial tumor challenge, with or without dexamethasone. Sixty percent of mice treated with IL-2 ($P < 0.001$ vs. control) were LTS of >120 days. Of the mice receiving daily injections of 1 mg/kg of dexamethasone, 54% were LTS of >120 days ($P < 0.005$). In contrast, there was no difference in median survival or overall survival in mice treated with 10 mg/kg of dexamethasone or control animals.

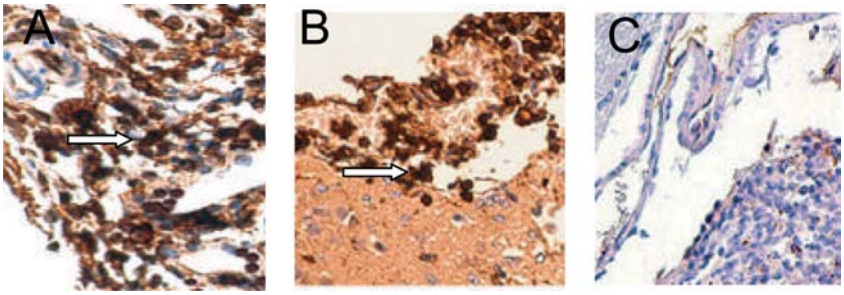

Figure 2. Immunohistochemical analysis of animal brains stained for the presence of the T cell marker, CD8. (A) Animals treated with hIL-2 showed the presence of CD8 cells (arrow). (B) Administration of dexamethasone at a dose of 1 mg/kg had no effect on the presence of the infiltrate whereas (C) 10 mg/kg was associated with minimal to no infiltrate ×300.

In order to better understand the mechanism of this response, we analyzed the brains of animals treated in this study. It has been previously shown by our group and others that local immunotherapy with IL-2 is associated with a CD8+ infiltrate within the tumor [3,5,8,9]. In this study, we have shown that the relative degree of this inflammatory response is significantly decreased in the presence of high dose dexamethasone and in some tissue samples, the lack of CD8+ infiltrate was comparable to that of controls. One likely mechanism that could explain this observation is the fact that IL-2 is responsible for clonal expansion of T cells. The presence of high dose steroids can interfere with T cell activation and proliferation, thereby down-regulating the expansion of CD8+ T cells. Indeed, previous studies looking at the role of glucocorticoids and T cells have shown a negative impact of steroids on T cell activity, including T cell apoptosis [14,16,18].

Another possibility rests in the immune function of resident CNS cells. One such cell, microglia, is primarily responsible for antigen processing and presentation within the central nervous system [19–22]. Recent studies, however, clearly illustrate that dexamethasone can exert negative effects on microglial function. For instance, Badie et al., have shown that even at low doses, dexamethasone

inhibits significantly the infiltration of brain tumors by microglia [18]. Similarly, Drew et al., have demonstrated that cortisol directly inhibits microglial cell activation [23]. In this setting, we can postulate that increasing doses of steroids can be associated with inhibition of microglial function and subsequent down-regulation of T cell expansion. However, until the results of these studies are complete, the precise mechanism by which increasing doses of dexamethasone is related to decreased CD8+ infiltration will remain open to speculation.

In summary, the results of our study provide new data to support and further encourage the development of immunotherapy protocols for patients with brain tumors. Careful and judicious use of steroids within this patient population is not likely to have a significant impact on IL-2 based immunotherapy. However, the true impact of steroids will have to await future immunotherapy trials. In the meantime, we propose that all studies involving immunotherapy should be performed with an appropriate control which takes into account the systemic administration of steroids to patients with malignant brain tumors.

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Address for offprints: Maciej S. Lesniak, The University of Chicago Hospitals, Neurosurgery, MC 3026, 5841 S. Maryland Avenue, Chicago, IL 60637, USA; Tel.: +1-773-702-2123; Fax: +1-773-702-3518; E-mail: mlesniak@surgery.bsd.uchicago.edu