Increasing the efficacy of antitumor glioma vaccines by photodynamic therapy and local injection of allogeneic glioma cells

Catherine E. Christie\textsuperscript{a}, Qian Peng\textsuperscript{b}, Steen J. Madsen \textsuperscript{c}, Francisco A. Uzal\textsuperscript{d} & Henry Hirschberg\textsuperscript{a,c}

\textsuperscript{a}Beckman Laser Institute and Medical Clinic, University of California Irvine.

\textsuperscript{b}The Norwegian Radium Hospital, Oslo University Hospital, Oslo Norway

\textsuperscript{c}Dept. of Health Physics and Diagnostic Sciences, University of Nevada, Las Vegas

\textsuperscript{d}School of Veterinary Medicine, University of California Davis, San Bernardino div., California

ABSTRACT

Immunotherapy of brain tumors involves the stimulation of an antitumor immune response. This type of therapy can be targeted specifically to tumor cells thus sparing surrounding normal brain. Due to the presence of the blood-brain barrier, the brain is relatively isolated from the systemic circulation and, as such, the initiation of significant immune responses is more limited than other types of cancers. The purpose of this study was to show that the efficacy of tumor primed antigen presenting macrophage vaccines could be increased by: (1) PDT of the priming tumor cells, and (2) injection of allogeneic glioma cells directly into brain tumors.

Experiments were conducted in an \textit{in vivo} brain tumor model using Fisher rats and BT\textsubscript{4}C (allogeneic) and F98 (syngeneic) glioma cells. Preliminary results showed that vaccination alone had significantly less inhibitory effect on F98 tumor growth compared to the combination of vaccination and allogeneic cell (BT\textsubscript{4}C) injection.

\textbf{Keywords:} brain tumor, glioma vaccine, immunotherapy, macrophages, photodynamic therapy, BT\textsubscript{4}C, F98

*steen.madsen@unlv.edu; phone: 702.895.1805; Fax: 702.895.4819

1. INTRODUCTION

Malignant gliomas are the most common primary brain tumors in adults. The primary treatment for high grade gliomas is surgical resection. This is typically followed by secondary treatments consisting of radiation and chemotherapy that aim to eradicate islands of tumor cells that can extend into the surrounding brain tissue. As evidenced by the high recurrence rates, predominantly at the original resection site, standard glioma treatment regimens are rather ineffective suggesting the need for improved therapies such as the development of tumor vaccines. The advantage of generating an immune response towards a cancer is that the immune effectors can now seek out and destroy the tumor cells that are located in inaccessible sites that traditional surgery, radiation, or chemotherapeutic drugs cannot reach. Patients diagnosed with malignant brain tumors have an average survival time of 12-18 months. Immunotherapy treatment for brain tumors involves the stimulation of an antitumor immune response\textsuperscript{1}. This type of therapy can be specifically targeted to tumor cells, sparing the surrounding normal brain tissue since several glioma associated antigens have been demonstrated\textsuperscript{2}. Antigen presenting cell (APC)-based vaccine therapy for primary CNS tumors has been an area of active research, but further development of APC-based cancer vaccines is still necessary to make them a therapy with an improved efficacy and tolerability. Due to the presence of the blood-brain barrier, the brain is relatively isolated from the systemic circulation and, as such, the initiation of significant immune responses is more limited than other types of cancers. APC vaccine therapy can be accomplished using either dendritic cells (DC) or macrophages (Ma). The use of Ma cell lines as APC instead of DC...
greatly simplifies the work involved and reduces the number of animals required by half since no donor animals are required. Recent work has demonstrated that Ma and DC are equally active at presenting antigens.

This study is based on the hypothesis that the efficacy of tumor antigen primed Ma-mediated immunotherapy can be increased by: (1) Photodynamic therapy (PDT) treatment of tumor cells, and (2) injection of allogeneic glioma cells directly into brain tumors. PDT will generate multiple antigenic (tumor) peptides which can be used to activate APC such as macrophages (Ma) ex vivo. When these ex vivo activated APC are reintroduced back into the patient (or experimental animal), the host’s antitumor T cells are stimulated, and will subsequently attack the remaining glioma cells. Direct injection of allogeneic glioma cells into brain tumors will stimulate an immunological rejection reaction and will enhance the specific response in the tumor to the cell vaccine. Allogeneic glioma cells (BT4C) transplanted into the brain will be rejected via an immunological response directed to the foreign major histocompatibility complex (MHC) as well as tumor-associated antigens. Since T cells react to antigens presented and bound to self MHC (i.e. modified self MHC), these two reactions have many similarities. This is a proof-of-concept study in vivo using a brain tumor model in rats. Due to the disseminated character of brain tumors, activation of the immune system should be well suited for treatment of brain cancer.

2. MATERIALS AND METHODS

2.1 Cell lines

F98 glioma cells and rat alveolar Ma (NR8383) were obtained from the American Type Culture Collection (Manassas, VA). The BT4C tumor was originally derived from transformed fetal BD-IX rat brain cells after exposure to ethyl – nitrosourea. The cells were cultured in Dulbecco’s Modified Eagle Media (DMEM, Gibco, Carlsbad, CA) with high glucose and supplemented with 2 mM L-glutamine, gentamycin (100 mg/ml), and 2% heat-inactivated fetal bovine serum (Gibco) at 37°C in a 7.5% CO2 incubator.

2.2 PDT treatment and Ma loading.

F98 monolayers were incubated with AlPcS2a (1 µg/ml for 18 h). Following wash the cells were irradiated with 5J/cm² at λ = 670 nm. MTS assays showed less than 5% surviving cells. 1x10⁶ PDT treated F98 cells were cocultured with 0.5 x 10⁶ NR8383 Ma for 24 h. The combined cell mixtures were then used to inoculate the animals.

2.3 Animal model

The glioma model consisted of F98 tumor cells in Fisher rats. The protocol is illustrated in Fig. 1.
The study protocol consisted of 4 arms: (1) F98 injected control animals (no treatment), (2) BT4C injected animals (allogeneic control), (3) combination of injected F98 and BT4C, (4) F98+BT4C injected tumor cells into PDT primed Ma vaccinated animals.

F98 and BT4C cells were injected stereotactically into the brains of Fisher rats as previously described. Briefly, anaesthetized rats were fixed in a stereotactic frame. The skin was incised and a 1.0-mm burr hole was made at the following coordinates: 1 mm posterior to the bregma, 2 mm to the right of the midline and at a depth of 2 mm. The injection device consisted of a 30-G blunt cannula connected through a catheter (Hamilton Co., Reno, NV) to an infusion pump (Harvard Apparatus, Holliston, MA). The cannula was fixed in the electrode holder of the stereotactic frame, and then vertically introduced into the brain. A total of $10^4$ F98 and $10^5$ BT4C cells in 20 µl PBS were injected into the brain over a period of 2 min. Following injection, the cannula remained in place for 2 min. Closure was done with bone wax and sutures. The animals were followed for 14 days, euthanized and the brains removed. Histology of removed brains was performed with H&E staining.

### RESULTS

#### 3.1 Syngeneic and allogeneic controls

In order to reduce the number of animals required, arms 1 and 2 were done in the same animal, F98 in one (right) hemisphere and BT4C in the other (left). The results are shown in Fig. 2 for two typical animals. All of the animals developed F98 tumors while none developed BT4C tumors. This clearly demonstrated that allogeneic cells in the brain were rejected.
3.2 Combined injection of syngeneic and allogeneic cells

In arm 3, mixtures of F98+BT4C were injected together in the same hemisphere. Fig. 3 shows a typical result, i.e., all of the animals developed tumors, indicating the rejection reaction against the BT4C cells was insufficient to prevent F98 tumor growth.

3.3 Tumor cell injection into vaccinated animals

Animals were injected (i.p.) with PDT-treated F98-loaded Ma and two days later F98+BT4C cells were stereotactically injected into the brain. Although the animals developed tumors, they were significantly smaller than tumors in the non-treated controls (Fig. 4).
4. DISCUSSION AND CONCLUSION

Several approaches for immunotherapy for gliomas have been reported including: (1) passive immunotherapy with monoclonal antibody (mAb) treatment with stimulated cells (adoptive) and, (2) active specific immunotherapy where DC vaccines predominate. In order to stimulate an anti-tumor immune response, the immune system’s T cells must be presented with tumor cell antigens. T cell immune response involves three key components: CD8+ T cells that can target and kill the tumor cells in an antigen-specific manner, CD4+ T cells that can either “help” the generation of a productive CD8+ T cell or “regulate/suppress” it, and the antigen presenting cells (APC) that can efficiently process the antigens and present them to the effector T cells in small fragments, termed antigenic epitopes. Several glioma specific antigens have been reported. In general, both in experimental and in clinical trials, DC obtained from donor animals or directly from patients (by leukapheresis) have been used as APC. In the experiments reported here, macrophages were used as APC instead of DC. DCs have been considered an immune cell type that is specialized for the presentation of antigens to naive T cells. On the other hand, recent work has indicated that that DC are a part of the mononuclear phagocyte system (MPS) and that there are no pathways of development, markers, or even functions as APC that distinguish them from macrophages.

The use of Ma established cell lines as APC greatly simplified the experimental protocol used in this study. Besides using Ma as APC, PDT treatment of the priming tumor cells was also applied. PDT not only causes apoptotic and necrotic tumor cell death, but also has the potential to create an environment at the tumor site that favors both tumor antigen loading and activation of APCs, important components for induction of antitumor immunity. The combination of PDT-mediated active immunization together with an immunological tissue rejection reaction, as supplied by the allogeneic BT4C cells in this study, appears to be a logical approach with few side effects.

Figure 4. H&E-stained sections showing tumor development in non-treated controls and immunotherapy vaccinated animals. The treated animals were injected (i.p.) with PDT-treated F98-loaded Ma two days prior to stereotactic combined F98 and BT4C tumor cell implantation.
The preliminary results from this pilot study indicate that immunization with macrophages primed with PDT-treated tumor cells is capable of slowing the development of F98-induced tumors in the brain (Fig. 4). No side effects were noted in any of the animals during the 14-day period of observation. In the experiments reported here, the immunizing loaded macrophages were injected i.p. This is probably not an optimized immunization route. Work is in progress aimed at inducing a more efficient immunizing protocol. Direct injection of naive APC into a PDT-treated tumor is under investigation and has been shown to provide promising results in other forms of tumor models.

ACKNOWLEDGMENTS

This work was supported by grants from the Norwegian Radium Hospital Research Foundation. Portions of this work were made possible through access to the Laser Microbeam and Medical Program (LAMMP) at the University of California, Irvine. Steen Madsen is grateful for the support of the Tony and Renee Marlon Charitable Foundation.

REFERENCES


