Treatment of intracranial gliomas with bone marrow–derived dendritic cells pulsed with tumor antigens

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Object. An approach toward the treatment of intracranial gliomas was developed in a rat experimental model. The authors investigated the ability of “professional” antigen-presenting cells (dendritic cells) to enhance host antitumor immune responses when injected as a vaccine into tumor-bearing animals.

Methods. Dendritic cells, the most potent antigen-presenting cells in the body, were isolated from rat bone marrow precursors stimulated in vitro with granulocyte–macrophage colony-stimulating factor (GM-CSF) and interleukin-4. Cultured cell populations were confirmed to be functional antigen-presenting cells on the basis of expressed major histocompatibility molecules, as analyzed by fluorescence-activated cell sorter cytofluorography. These dendritic cells were then pulsed (cocultured) ex vivo with acid-eluted tumor antigens from 9L glioma cells. Thirty-eight adult female Fischer 344 rats harboring 7-day-old intracranial 9L tumors were treated with three weekly subcutaneous injections of either control media (10 animals), unpulsed dendritic cells (6 animals), dendritic cells pulsed with peptides extracted from normal rat astrocytes (10 animals), or 9L tumor antigen–pulsed dendritic cells (12 animals). The animals were followed for survival. At necropsy, the rat brains were removed and examined histologically, and spleens were harvested for cell-mediated cytotoxicity assays.

The results indicate that tumor peptide–pulsed dendritic cell therapy led to prolonged survival in rats with established intracranial 9L tumors implanted 7 days prior to the initiation of vaccine therapy in vivo. Immunohistochemical analyses were used to document a significantly increased perilesional and intratumoral infiltration of CD8+ and CD4+ T cells in the groups treated with tumor antigen–pulsed dendritic cells compared with the control groups. In addition, the results of in vitro cytotoxicity assays suggest that vaccination with these peptide-pulsed dendritic cells can induce specific cytotoxic T lymphocytes against 9L tumor cells.

Conclusions. Based on these results, dendritic antigen-presenting cells pulsed with acid-eluted peptides derived from autologous tumors represent a promising approach to the immunotherapy of established intracranial gliomas, which may serve as a basis for designing clinical trials in patients with brain tumors.

KEY WORDS • antigen-presenting cell • brain neoplasm • cytokine • dendritic cell • immunotherapy • tumor vaccine • rat

Despite advances in the understanding of tumor biology at the cellular and genetic level, the prognosis of patients with primary malignant glioma has not improved significantly over the past 20 years. Current treatments, including surgery, radiation therapy, and systemic chemotherapy, unfortunately have not changed the natural history of these incurable neoplasms. Therefore, the development of new therapies to treat malignant gliomas is essential.

An emerging strategy in the treatment of various neoplasms involves the stimulation of an immune response against the malignant cells. Among the new treatments currently being investigated for malignant cancers, immunotherapy is theoretically very appealing, because it offers the potential for high tumor-specific toxicity. However, there is concern about the applicability of immunologically based therapies to central nervous system (CNS) tumors. One aspect of this is that the CNS is immunologically privileged; it is devoid of lymphoid reactivity and normal immune surveillance. Furthermore, it has been observed in several different laboratories that effective anti–CNS tumor immune responses can be generated using cytokine-modified tumor cell vaccines. Therefore, the possibility that the immune system can mediate interactions with lesions in the CNS (such as brain tumors) presents excellent opportunities to investigate immunological modes of therapy.
Recent advances in the understanding of antigen presentation, antigen recognition requirements, and T-cell activation have centered around dendritic cells as a novel form of immunotherapy for the treatment of cancer.1,2,16,23,24,32–34,39,40 Dendritic cells are the most potent “professional” antigen-presenting cells in the body. Research evidence indicates that, although tumors may contain immunogenic antigens, tumor cells themselves are poor antigen-presenting cells. Therefore, professional antigen-presenting cells may be needed to internalize, process, and/or present tumor antigens to T cells efficiently.11,20

Dendritic cells are derived from proliferating bone marrow precursor populations. Investigators have recently discovered that large numbers of functional dendritic cells can be isolated from bone marrow precursor cells in vitro with the support of certain cytokines. Granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-4 (IL-4) induce progenitor cell differentiation into dendritic cells, whereas tumor necrosis factor–α inhibits granulopoiesis and maintains the viability of dendritic cells in culture.14 In mouse models and in clinical trials, these cytokine-stimulated dendritic cells have been successfully pulsed ex vivo with tumor antigens for use as antitumor vaccines against cancers outside the CNS.25,33,39,40 In the present study we report the use of dendritic cell immunotherapy for the treatment of brain tumors within the CNS.22,23

Materials and Methods

Dendritic Cell Isolation

Dendritic cells were harvested from syngeneic rat bone marrow. Nine Fischer 344 rats were killed, and their femurs and tibias were cleaned of all muscle tissues by using a gauze pad. The bones were placed in 70% alcohol, washed twice with phosphate-buffered saline (PBS), and transferred into a clean Petri dish. Both ends of the bones were cut with sterile scissors, and the marrow was flushed out using a 20-gauge needle and syringe with 5 ml of RPMI-1640 culture medium. The tissue was then suspended in culture medium and passed through nylon mesh to remove any bone or debris. These bone marrow suspensions were then supplemented with GM-CSF (1000 IU/ml medium) and IL-4 (1000 IU/ml medium) and incubated for 8 days. The addition of GM-CSF and IL-4 to cell cultures has previously been shown to induce differentiation of functional dendritic cells from bone marrow progenitors.1,4,17,22,27,29,37,39 Cultured cells were identified as functional dendritic cells based on microscopically confirmed morphological features and the expression of cell surface antigens, as identified using cytofluorographic analysis. On the basis of earlier publications, the markers chosen to identify dendritic cells positively were OX62+ (an integrin molecule specifically expressed on rat dendritic cells) and OX6+ (a rat nonpolymorphic determinant of MHC Class II antigens).20 Negative selection was confirmed using lymphocyte markers CD3, CD4, and CD8.

Tumor Peptide Pulsing

Prior to use in the animal experiments, some of these antigen-presenting dendritic cells were pulsed with soluble peptides. For the pulsed dendritic cell immunizations, the cells were cocultured overnight (16–24 hours) with 80 to 100 μg of acid-eluted peptides from either syngeneic 9L rat glioma cells or normal astrocytes cultured from the whole brains of Fischer 344 rats. Expression of dendritic cells to normal brain peptides served as a control for the tumor specificity of our treatment in the 9L cell line. For rapid isolation of cell-associated peptides, 108 9L glioma cells or normal astrocytes were washed three times with Hank’s buffered saline solution. The cell pellets were then treated with 5 ml of citrate-phosphate buffer (pH 3.3), and the pellets were immediately resuspended by pipetting and centrifuged for 5 minutes at 1000 G. The cell-free supernatant was harvested, and peptides in the acid-extracted supernatant were concentrated in activated elution columns. The bound material was eluted with 3 ml of 60% acetonitrile in water and lyophilized to nearly complete dryness (20–50 μl). The peptides were reconstituted in 1 ml of PBS and adequate yields were confirmed using standard Bradford protein assays. The average protein yields from this acid-elution technique were found to be 80 to 100 μg per 108 cells.

In Vivo Animal Experiments

The tumor cell line designated 9L is a type of gliosarcoma that originated in Fischer 344 rats. These cells were maintained in routine tissue culture in Hams F-12 medium (Gibco-BRL), supplemented with 10% fetal bovine serum, and antibiotic drugs (100 U/ml penicillin and 100 μg/ml streptomycin) at 37°C in a 5% CO2/95% air atmosphere. Tumor cells (106) were then injected intracranially into 5- to 6-week-old syngeneic Fischer 344 rats. Intracranial
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tumor implantation was performed with the aid of stereotactic guidance. Two mm anterior and 2 mm lateral to the junction of the bregma and sagittal sutures. Thirty-eight animals were used for these experiments, and all were maintained and treated according to the University of California at Los Angeles Animal Research Committee guidelines.

One week after tumor implantation, 12 animals were treated with three weekly subcutaneous flank injections of 5 × 10^6 syngenic 9L antigen-pulsed dendritic cells, six were treated with 5 × 10^6 unpulsed dendritic cells, 10 with 5 × 10^6 dendritic cells pulsed with soluble peptides extracted from normal rat astrocytes, and 10 with control media (Fig. 1). The vaccination schedule was empirically chosen based on previous observations that three injections of dendritic cells 1 week apart conferred optimal antitumor immunity in tumors outside of the CNS (JS Economou, personal communication, 1997). Animals in each of the treatment groups were followed for survival.

Histopathological and Immunohistochemical Examination of Tumors

Each animal’s brain was removed at necropsy for histological and/or immunohistochemical examination. First, the rats were perfused with 500 ml of saline followed by 750 to 1000 ml of 4% paraformaldehyde. Their brains were then removed and postfixed overnight in 4% paraformaldehyde at 4°C. The brains were stored at 4°C in 15% sucrose in PBS for 2 days before cryosectioning. Slices were cut by means of a cryostat through the area of tumor implantation at a thickness of 20 μm, and the sections were either mounted on glass slides for routine histological staining with hematoxylin and eosin or frozen in PBS for immunohistochemistry.

For the immunohistochemical analysis, 20-μm sections were incubated overnight at 4°C with mouse monoclonal antibodies directed against rat CD8, CD4, and Pan-T markers, or macrophages. After buffer washes, species-absorbed biotinylated goat anti-mouse secondary antibody F(ab')2 was applied to sections incubated with unlabeled primary antibody, followed by treatment with streptavidin horseradish peroxidase for 30 minutes at room temperature according to the manufacturer’s protocol. Finally, immunoreaction products were visualized by incubation in a solution of 0.02% 3,3'-diaminobenzidine tetrahydrochloride and 0.005% H2O2 in PBS at pH 7.6. Sections were counterstained with thionine and mounted on a coverslip. For each pair of rat brains analyzed (control compared with treated), all reactions were performed at the same time by using the same reagents. Staining for CD8, CD4, and Pan-T lymphocyte markers, as well as macrophages, was graded in a blinded manner in each specimen and scored as none, minimal, moderate, or heavy.

In Vitro Cytotoxicity Assay

Spleens were removed at necropsy in groups of rats treated with either control media or 9L peptide-pulsed dendritic cells. Splenocytes were cultured in RPMI-1640 with 10% fetal calf serum, 1% L-glutamine, 100 IU/ml penicillin, 100 mg/ml streptomycin, and 0.05 mM β-mercaptoethanol in 24-well flat-bottom plates. After 5 days in culture, the viable splenocytes were used as effector cells in standard 4-hour europium release assays to determine specific cell lysis against 9L target cells.23

Concentrations of 5 × 10^6 9L target cells were loaded with europium diethylene-triamine pentaacetate for 20 minutes at 4°C. After the incubation, 30 μl of 100 mM CaCl2 was added to seal the cells, and after mixing, these were left at 4°C for another 5 minutes. The cells were then washed three times with RPMI-1640. Afterward, 10 μl europium-labeled 9L target cells and serial dilutions of effector cells at various effector/target ratios were incubated for 4 hours in 200 μl of RPMI 1640 with 10% heat-inactivated fetal calf serum in 96-well V-bottom plates at 37°C with 5% CO2. After incubation, the plates were centrifuged at 600 G for 5 minutes, and 50 μl of the supernatant was harvested from each well and transferred to new 96-well flat-bottom plates. Europium release was measured by time-resolved fluorescence using a Delta fluorometer. The percentage of specific release was calculated using the following formula: (experimental release − background release)/(maximum release − background release) × 100. Spontaneous release was noted to be less than 25%. The standard error of the means of triplicate cultures was less than 5%.

Statistical Analysis

Survival estimates and median survival times were determined using the Kaplan–Meier method. Survival data were compared using Wilcoxon’s log-rank test. Two-sided probability values (Student’s t-test) were calculated for the other data. Statistical significance was set at the level of p < 0.05.

Sources of Supplies and Equipment

The RPMI-1640, fetal calf serum, Hank’s buffered saline solution, and Hams F-12 medium were purchased from Gibco-BRL, Gaithersburg, MD. The GM-CSF, β-mercaptoethanol, and diethylenetriamine pentaacetate were acquired from Sigma Chemical Co., St Louis, MO. The IL-4 was purchased from Genzyme, Cambridge, MA, and the lymphocyte markers CD3, CD4, CD8, and Pan-T were provided by Pharmingen, San Diego, CA. The syngeneic 9L rat glioma cells were purchased from American Type Culture Collection, Rockville, MA. The SepPark C18 elution columns were acquired from Millipore Corp., Bedford, MA. The streptavidin horseradish peroxidase was purchased from Vector Labs, Burlingame, CA; the 3,3'-diaminobenzidine tetrahydrochloride from Pierce, Rockford, IL; the L-glutamine from Irvine Scientific, Santa Ana, CA; and the CaCl2 from Merck, Yonkers, NY. The Delta fluorometer was obtained from Wallace, Inc., Gaithersburg, MD. The FACScan for cytofluorographic analysis was purchased from Becton Dickinson and Co., San Jose, CA.

Results

Isolation and In Vitro Characterization of Rat Bone Marrow–Derived Dendritic Cells

Cytospin preparations of dendritic cell–enriched bone marrow cultures were examined daily for the first 8 days in culture. Representative photomicrographs of the observed cells are shown in Fig. 2. Day 1 cytospin preparations contained a large variety of cell types, which were very similar to those seen on smears of unfractionated bone marrow aspirates. Cells belonging to both the myelomonocytic and normoblastic lineages were present. By Day 3, cells in the normoblastic series had largely disappeared from the cultures, whereas the myelomonocytic elements had increased proportionately. The immature myeloid cells formed clusters, some of which had morphological features of the myelomonocytic lineage (Fig. 2A). During Days 5 and 6, dendritic-like cells appeared and became more mature, showing characteristic irregular nuclei and smooth, blue–gray cytoplasm with short cytoplasmic dendrites (Fig. 2B). By Day 8, fully mature dendritic cells with typical cloverleaf-shaped nuclei and long cytoplasmic dendrites appeared (Fig. 2C and D).

Functional dendritic cells were also characterized on the basis of their expression of certain cell surface markers. The markers chosen to identify positively dendritic cells were OX62+ and OX6+. OX62 is an integrin molecule specifically expressed on functional rat dendritic cells, and OX6 is a rat nonpolymorphic determinant of MHC Class II molecules. These markers were chosen on the basis of prior publications.7,8 Although the MHC Class II+ marker labels some of the OX62+ cells, true dendritic cell populations are MHC Class II+ and OX62+.
Because OX62 has some crossover recognition of CD3+ γδ T cells with dendritic morphology, T-lymphocyte markers (CD3, CD4, and CD8) were used as negative identifiers for dendritic cells. These lymphocytic antigens were not detectable in our cultured dendritic cell populations (CD3+/H11002, CD4+/H11002, and CD8+/H11002), confirming the relative purity of our MHC Class II+/OX62+/CD3+/H11002 dendritic cell cultures. All these markers were studied using fluorescence-activated cell sorter cytofluorography. Dendritic cells were gated out (Fig. 3A) on the basis of high forward light scattering (size) and low side scattering (density), which included 40 to 52% MHC Class II positive cells in the preselected bone marrow cultures at Day 4 (Fig. 3B) and 86 to 94% in the mature dendritic cell cultures at Day 8 (Fig. 3C).

**Survival of Rats With Intracranial 9L Gliomas After Treatment With Tumor Antigen–Pulsed Dendritic Cells**

The survival of intracranial tumor-bearing rats injected subcutaneously with the syngeneic bone marrow–derived dendritic cells pulsed (cocultured) with acid-eluted tumor peptides was significantly prolonged relative to the survival of rats receiving equivalent numbers of dendritic cells pulsed with control (normal rat astrocyte) peptides, unpulsed dendritic cells, or control media alone (p < 0.05). Seven (58%) of 12 animals treated with tumor antigen–pulsed dendritic cells were alive at 31 days, whereas none (0%) of the six animals treated with unpulsed dendritic cells, or of the 10 animals treated with control peptide-pulsed dendritic cells, or of the 10 animals treated with control media survived past 31 days. Rats treated with control media, unpulsed dendritic cells, or control peptide-pulsed dendritic cells had median survival times of 16, 17, and 22 days, respectively. In contrast, rats in the 9L antigen–pulsed dendritic cell–treated group had a significantly longer median survival time of 35 days (p = 0.027). When the experiment was extended to 60 days, three (25%) of the 12 9L antigen–pulsed dendritic cell–treated rats were found to be long-term survivors (Fig. 4).

**Histological Characterization of Intracranial Tumors**

The brains from each of the animals in the different treatment groups were removed soon after death for histopathological and immunohistochemical examination. The tumors ranged in size from 125 to 1000 mm³. In specimens from all groups, the brain parenchyma outside the immediate peritumoral regions appeared normal on histological examination, with no appreciable inflammatory infiltration or demyelination. There was no evidence of experimental allergic encephalitis induced by the dendritic cell–based vaccine therapy, either in the animals that died early (Fig. 5A–C) or in the long-term survivors (Fig. 5D and E).

Analysis of intracranial tumor volumes was performed in all rats that died within 21 days postimplantation. Seven of the animals that received control media, four of those given unpulsed dendritic cells, four of those given normal peptide-pulsed dendritic cells, and three treated with the...
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9L antigen–pulsed dendritic cells were dead at 3 weeks. In the animals that died early, there were no significant differences in the mean tumor size at death among the four experimental groups (data not shown). This further supports the observation that, compared with the control animals, the animals treated with dendritic cells that died early probably died of their intracranial tumor load and not of any unusual immune inflammation. The brains of the three animals treated with 9L antigen–pulsed dendritic cells that survived past 60 days revealed no evidence of tumor, suggesting eradication of established lesions in these long-term survivors (Fig. 5D and E).

Immunohistochemical analysis of five animals from each group documented an increased perilesional and intratumoral infiltration of CD8+ T cells (and, to a lesser extent, CD4+ T cells and macrophage cells) in the group treated with antigen-pulsed dendritic cells compared with controls (Fig. 6). Tumors from all five animals analyzed in the group treated with 9L antigen-pulsed dendritic cells were associated with moderate to heavy T-cell infiltration, whereas no intratumoral T-cell infiltration was observed in any of the similarly stained brains examined from the control group.

Induction of Tumor-Specific Cytotoxic T Lymphocytes

To determine whether the prolonged survival seen in our rats treated with antigen-pulsed dendritic cells was actually a result of the induction of tumor-specific cytotoxic T-lymphocyte (CTL) immune responses in vivo, we performed standard in vitro europium cytotoxicity assays by using splenocytes harvested from animals in each of the experimental groups. Cytotoxic activity was tested against irradiated 9L tumor cells. Because the animals in our survival study died on different days, five separate cytotoxicity assays were performed. As demonstrated in Fig. 7, three of five of the cytotoxicity assays showed a trend in which animals immunized with tumor peptide–pulsed dendritic cells were capable of eliciting 9L-specific CTL responses that were statistically significant compared with animals immunized with control media (Fig. 7C–E). Two of the assays that we performed revealed no significantly increased CTL response in the treated compared with control animals (Fig. 7A and B).

Discussion

In this study, we used two very stringent experimental systems to assess the efficacy of our proposed vaccine: 1) prolongation of survival in tumor-bearing animals in vivo; and 2) induction of a primary CTL response in vitro. The results of our in vivo studies showed that treatment of tumor-bearing rats with dendritic cells pulsed with acid-eluted tumor peptides led to a dramatic reduction in the mortality rate at 31 days. At this juncture, 58% of our treated animals had survived, whereas all of our control animals had died of their intracranial tumors. At 60 days, 25% of the treated animals were still alive, and brain histological analysis indicated that our tumor peptide–pulsed
dendritic cell therapy led to the eradication of established tumors in these long-term survivors. Despite the significant survival advantage afforded by our dendritic cell–based therapy, additional studies are needed to improve the therapeutic efficacy of this treatment strategy. Ideally, an effective antitumor vaccine should stimulate a strong and enduring immune response, which translates to complete and consistent eradication of tumor. In this study, complete tumor eradication may have been limited by the "immunological privilege" of the blood-brain barrier, the inherent immune variability among the rats tested, or the use of unfractionated tumor peptides instead of a purified tumor-specific antigen. One possible drawback of the use of unfractionated tumor peptides is the potentially low concentration of effective tumor antigens in the mixture. Antigenic tumor peptides may conceivably be diluted by relatively nonantigenic proteins, which could lower the effectiveness of the antitumor immune response. This is a possibility that needs to be further evaluated. Nevertheless, the in vivo data demonstrated in this study underscore the ability of peptide-pulsed dendritic cells to prolong survival effectively in animals with intracranial tumors, and further studies to optimize dendritic cell–based vaccines are warranted.

The results of our in vitro cytotoxicity assays are also highly suggestive of the ability of peptide-pulsed dendritic cells to express specific tumor antigens and induce an immune response against intracranial tumors. Induction of a primary CTL response is one of the most stringent tests of antitumor immunity. As presented here, three of the five cytotoxicity assays showed that animals treated with peptide-pulsed dendritic cells showed a significantly increased primary anti-tumor CTL response in vitro (Fig. 7C–E) compared with controls. Although we cannot directly account for the lack of an impressive CTL response seen in two of the assays (Fig. 7A and B), we speculate that the variability of results among the different assays may be caused by inherent differences in the immune responses of the individual animals tested during each run. Interestingly, the two assays that showed no significant difference in CTL response between treated and control animals (Fig. 7A and B) were performed using splenocytes from rats that had died early in the survival experiment. Perhaps the animals treated with dendritic cells in this group never mounted a specific immune response against their tumors, which may explain why they died at approximately the same time as their untreated counterparts. This observation may further support the hypothesis that induction of an antitumor CTL response in vitro translates into prolonged survival in vivo.

Based on this and other studies, the concept of using tumor peptide–pulsed antigen-presenting cells (dendritic cells) as a means to elicit antitumor immunity is an attractive method of therapy. To our knowledge, only two other studies of dendritic cell–based therapy for CNS tumors have been published previously. One used whole-tumor extracts and total RNA to pulse dendritic cells, whereas the other used a synthetic viral peptide to elicit antitumor immunity. The novelty of our study lies in the use of acid-eluted glioma surface peptides in conjunction with dendritic antigen-presenting cells to serve as a vaccine for established intracranial tumors.

This protocol strategically addresses several of the current limitations of brain tumor immunotherapy. For instance, although dendritic cells are ubiquitous in the body, they comprise less than 0.1% of circulating white cells in human blood. Until recently, their expansion in large numbers ex vivo has been difficult, and many investigators have resorted to stimulating the growth of these antigen-presenting cells in vivo with the use of soluble cy-
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Fig. 5. Photomicrographs showing representative hematoxylin and eosin–stained sections from tumor-bearing rats treated with 9L peptide–pulsed dendritic cells. A–C: Brain sections from an experimental animal that died early (21 days after tumor implantation). Low-power view (A) shows a large intracranial tumor mass. Higher-power views (B and C) of the boundary between normal brain tissue and tumor. Note the lymphocytic accumulation within the tumor but no significant inflammation outside the immediate peritumoral area. The normal brain tissue is marked by an asterisk, and the tumor is marked by a T. D and E: Brain sections from an experimental animal that was a long-term survivor (> 60 days postimplant). Low-power view (D) shows absence of tumor at the implantation site. Higher-power view (E) shows some small areas of necrosis but no appreciable inflammatory infiltrate within the brain parenchyma. Original magnifications × 20 (A); × 200 (B); × 400 (C); × 20 (D); × 200 (E).

1. The isolation of large numbers of functional antigen-presenting dendritic cells ex vivo, however, may conceivably bypass the need to stimulate their in vivo differentiation with high-dose (potentially toxic) cytokine administration or cytokine gene therapy.

Another promising feature of our approach to brain tumor immunotherapy is the use of acid-eluted tumor peptides, which was first described by Zitvogel, et al. The use of tumor peptides for antigen presentation circumvents the potential risks associated with whole-cancer-cell vaccines, whether genetically engineered or not. Thus, peptide-based vaccines are beginning to take center stage in several investigational studies. The results of a recent study by Okada, et al. showed long-term survival in 67% of mice with intracranial C3 tumors that were treated with dendritic cells pulsed with a strongly immunogenic synthetic peptide antigen. However, unlike the current study, this previous investigation used tumor cells that were genetically transformed to express a foreign antigen, rather than targeting naturally occurring surface antigens as in our acid-eluted peptide model. Although some genes encoding tumor-associated antigens recognized by CD8+ T cells have recently been cloned (that is, for melanoma, lymphoma, and prostate cancer), the vast majority of the histological subtypes of CNS cancers express tumor epitopes that are as yet undefined. Since the advent of monoclonal antibody technology, it has been suggested that there are few true tumor-specific antigens. Therefore, without a relatively specific tumor-associated antigen...
identified for human gliomas, targeting a single tumor-specific peptide may not be entirely realistic in the clinical setting.

Furthermore, it has been suggested that an optimum host antitumor T-cell response against certain cancers may require a broad spectrum of epitopes rather than responses restricted to a single tumor–associated determinant. If this proves to be the case, acid elution of peptides from glioma cell surfaces or use of tumor total RNA may be better techniques for obtaining immunogenic epitopes than single-antigen approaches. Previous studies have shown that the method we used for eluting tumor-derived epitopes effectively extracts peptides bound to MHC Class I molecules on tumor cell surfaces and does not result in significant cell lysis. This has been proven to yield a superior source of immunogenic epitopes compared with peptides extracted from cell lysates, which presumably results in a more complex mixture of nonimmunogenic peptides and intracellular proteases. Another attractive alternative to the use of cell lysates is the use of RNA from tumor cells as a source of antigen. It has been reported in recent studies that RNA-pulsed dendritic cells are also effective in inducing antitumor immunity in vitro and in vivo. Ashley, et al., recently reported a 28.5% long-term survival rate in mice bearing CNS B16 tumors treated with pulsed dendritic cells, which is comparable with the 25% long-term survival rate found in our study. Therefore, along with other recently published studies, our current work provides further proof of the concept that effective CNS antitumor immunoreactivity can be generated with dendritic cell–based tumor vaccines.

Further comparisons between the immunogenic efficacy of dendritic cells pulsed with undefined multiple epitopes (that is, from acid-eluted glioma surface peptides or tumor RNA) and a defined single tumor–specific epitope will need to be evaluated if and when a glioma-specific antigen is identified. Meanwhile, our present method for acid extraction of MHC Class I–bound peptides from glioma cells appears to be a feasible and effective technique for antigen isolation in brain tumors.

Conclusions

Antigen presentation is a crucial factor in the induction of antitumor immune responses against malignant neoplasms. Dendritic cells are the most potent antigen-presenting cells for priming T cells in vivo and in vitro. The data presented here raise the possibility that injection of dendritic cells cocultured with acid-eluted glioma antigens can specifically activate antitumor T cells, which can infiltrate intracranial tumors and lead to significantly prolonged survival in tumor-bearing animals. This may therefore be a useful approach in the future treatment of malignancies of the CNS in humans.

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Fig. 7. Graphs showing induction of specific CTL lytic activity against 9L tumor cells in vitro by immunization with dendritic cells (DC) pulsed with acid-eluted tumor peptides. Tumor-bearing Fischer 344 rats were immunized three times with either dendritic cells pulsed with acid-eluted 9L peptides or control media. At necropsy, spleens were harvested and splenocytes were restimulated with irradiated 9L cells in vitro. Cytotoxic activity was then measured using a europium release assay as described in Materials and Methods. This experiment was repeated five times, with splenocytes from two to three rats per assay. The results of each of the five assays are shown in separate graphs. Error bars indicate 1 standard deviation of the mean. Statistical significance between the 9L peptide–pulsed dendritic cell–treated and control media–treated groups based on paired Student’s t-tests are as follows: p = 0.39 (not significant [A]); p = 0.50 (not significant [B]); p = 0.037 (C); p = 0.006 (D); p < 0.001 (E).

References