OBJECTIVE: On the basis of recent studies indicating that tumoral apoptotic bodies may provide a potent source of antigen for delivery to antigen-presenting cells, as well as observations that signal transduction modulation may constitute a promising approach for inducing glioma cell apoptosis, we explored the efficacy of vaccination with glioma apoptotic body-pulsed dendritic cells (DCs) for inhibiting tumor growth in the syngeneic 9L glioma/Fischer rat model.

METHODS: For induction of apoptosis, 7-hydroxystaurosporine (UCN-01) (200–300 ng/ml), a selective protein kinase C inhibitor, was co-incubated with 9L cells in vitro for 72 or 96 hours. After this pretreatment period, glioma cells and DCs were mixed, and the interaction between DCs and apoptotic 9L tumor cells was assessed using two-color flow cytometry. In a series of experiments, the efficacy of vaccination strategies using DCs co-cultured with apoptotic 9L cells was then examined in animals harboring intracranial tumors.

RESULTS: Pretreatment of 9L cells with UCN-01 resulted in approximately 50% of cells’ being observed to undergo apoptosis as compared with less than 3% of controls. After subsequent co-culture, two-color flow cytometry demonstrated a time-dependent physical association of DCs with the apoptotic glioma cells. Survival in animals harboring intracranial tumors was significantly longer for the animals treated with a glioma apoptotic body-pulsed DC vaccine than in the animals that received apoptotic glioma cells and DCs alone or vehicle (i.e., the controls), especially those that underwent a sequential vaccination strategy ($P < 0.0001$). Long-term survival (>90 d) was demonstrated in 6 (75%) of 8 animals that underwent this vaccination approach versus 0 (0%) of 16 controls. In contrast, no survival benefit was observed in animals that received DCs that were co-cultured with vehicle-treated (nonapoptotic) 9L cells. Three of four long-term survivors that were rechallenged intracranially with tumor cells also survived over the long term.

CONCLUSION: These studies suggest that induction of apoptosis in glioma cells by use of UCN-01 may promote the uptake of tumor antigens by DCs. This finding is important because apoptotic body-stimulated DCs may hold promise in promoting a host response against an established intracranial glioma, particularly if the parameters for apoptotic induction, duration of co-culture, and vaccination can be optimized.

Key words: Apoptosis, Dendritic cell, Glioma, Immunotherapy, Vaccine
The prognosis for patients with malignant gliomas has improved minimally during the past 30 years, despite advances in surgical, radiotherapeutic, and chemotherapeutic treatment modalities (10, 18, 23, 35). The poor results and significant morbidity associated with conventional treatment approaches for these tumors have provided an impetus for evaluating alternative therapeutic modalities that are more effective and capable of selectively targeting tumor cells. A significant focus of these research efforts has involved attempts to use cellular and humoral immune mechanisms to directly and specifically kill tumor cells (5, 17, 25, 26, 32, 39). The brain, and particularly brain tumors, however, present many challenges to successful immunotherapy that in part have accounted for the disappointing results of early immunotherapeutic approaches for patients with these tumors (11, 14, 20, 21).

Recent studies in other tumor systems have helped to define the parameters that are critical for the initiation of a productive immune response (13, 19, 28, 37). A central element in this process involves the presentation of tumor antigens in the appropriate context by so-called antigen-presenting cells to T-lymphocytes, which can then infiltrate and potentially attack the tumor. Dendritic cells (DCs), which are the most potent antigen-presenting cells, have therefore emerged as a promising component of vaccination strategies designed to promote a host response against a tumor target. A variety of strategies have been used for antigen delivery to DCs, including “pulsing” with tumor cell lysate/extract, tumor deoxyribonucleic acid and ribonucleic acid, and tumor-specific peptides (4, 17, 19, 26). One approach that has seemed particularly promising in recent studies with various solid tumor models has involved the delivery of tumor cell apoptotic bodies, which provide a readily available source of tumor antigens for uptake, processing, and presentation by DCs (2, 3, 8). However, the applicability of this approach for central nervous system tumors has not been reported.

Given the challenges involved in initiating a productive immune response against a central nervous system tumor target, we explored the applicability of a vaccination strategy that used glioma apoptotic body-pulsed DCs for inhibiting the growth of established intracranial tumors in a syngeneic rodent glioma model system. Apoptosis was induced by pretreating rat 9L gliosarcoma cells with 7-hydroxystaurosporine (UCN-01), a selective protein kinase C inhibitor, which was demonstrated in gliosarcoma cells with 7-hydroxystaurosporine (UCN-01), a selective protein kinase C inhibitor (6). In the current study, we observed that apoptosis induction facilitated glioma antigen uptake by DCs and, more important, that these apoptotic body-pulsed DCs were capable of delaying and in many cases preventing the growth of intracranial gliomas.

MATERIALS AND METHODS

Cell culture

Rat 9L gliosarcoma cells were maintained in minimal essential medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin G, 100 U/ml streptomycin sulfate, and 2.5% l-glutamine. Cells were cultured from passages 25 to 35 in 75-cm² flasks. Culture media were changed twice per week. Cells were incubated at 37°C in 5% CO₂ and subcultured weekly. For implantation, cells were washed in Hanks’ balanced salt solution (HBSS), detached in 1 ml 0.25% trypsin, collected by centrifugation (2000 x g for 7 min), and resuspended in HBSS.

Bone marrow-derived DCs were harvested from the femurs and the tibias of female Fischer 344 rats (National Cancer Institute, Charles River Laboratories, Frederick, MD). Cells were cultured as described previously with slight modification (9, 36). Before plating in 75-cm² flasks at a density of 4 x 10⁵ cells/ml, red blood cells were depleted using lysis buffer. Culture medium was composed of RPMI 1640, 2 mmol/L L-glutamine, 50 µg/ml gentamicin, 10 mmol/L N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid, 0.5 mmol/L N-methyl arginine, 10% fetal bovine serum, 0.1 mmol/L nonessential amino acids, 0.05 mmol/L L-mercaptoethanol, supplemented with recombinant murine granulocyte-macrophage colony-stimulating factor (1000 U/ml), interleukin-4 (1000 U/ml), and flt-3 ligand (75 ng/ml). Flt-3 ligand has been observed by our group (CS Brissette-Storkus, JC Kettel, T Witham, KM Giezeman-Smits, LA Villa, WH Chambers, submitted for publication) and by others (7, 15, 40) to enhance DC recovery in culture. Cytokines were replenished by exchanging half of the media every 2 days. Day 8 DCs were harvested from flasks by gentle pipetting of the nonadherent and loosely adherent cells. Rat DC phenotype was confirmed by staining cells with antibodies directed against the major histocompatibility complex class II antigen (OX-6), a rat myeloid cell marker, OX-42, and an integrin, OX-62, followed by two-color flow cytometry.

Assay for physical interaction between DCs and UCN-01-treated apoptotic 9L glioma cells

To examine the effect of tumor cell apoptosis on the uptake of glioma antigens by DCs, 9L cells were treated in vitro with 200 ng/ml of UCN-01 for 72 hours. At this point, approximately 50% of the glioma cells exhibited evidence of apoptosis as assessed by terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end-labeling assay (34), as compared with less than 5% of vehicle-treated cells, in agreement with our previously reported results (6). The glioma cells were then washed and labeled with PKH26 (16), a membrane-incorporated fluorescent marker. Syngeneic bone marrow-derived DCs were then admixed with UCN-01-treated 9L cells using a DC-to-tumor ratio of 5:1, and association between these two cell populations was evaluated immediately after mixing the cells and after in vitro co-culture at 37°C for 24 hours. Two-color flow cytometry was used to identify uptake and/or association of the PKH26-labeled glioma cells by the DCs, which were labeled by use of fluorescein isothiocyanate-conjugated OX-6 (labels major histocompatibility complex class II).

Assessment of vaccination strategies in an intracranial 9L gliosarcoma model

The efficacy of DCs co-cultured with UCN-01-treated (apoptotic) 9L glioma cells as a vaccine strategy was assessed in
an intracranial 9L model using a series of experimental conditions. The Animal Research and Care Committee of the Children's Hospital of Pittsburgh approved all animal studies. In these experiments, 4- to 6-week-old female Fischer 344 rats (Charles River Laboratories) were used.

For intracranial tumor injection, rats were anesthetized with ketamine (100 mg/kg) and acepromazine (0.02 mg/kg) and immobilized in a stereotactic frame. Via a small right paramedian scalp incision, an opening was made in the frontal bone by use of an 18-gauge needle tip. Stereotactic injection of 1 × 10^4 9L cells in a volume of 3 μl HBSS was directed into the right frontal lobe by use of a Hamilton syringe at a depth of 3 mm. For subsequent subcutaneous vaccinations, cells were suspended in 100 μl of a 1:1 mixture of phosphate-buffered saline/Matrigel (BD Biosciences, San Jose, CA) and injected into the right or the left flank. Details of the vaccination approaches used are described below.

The animals harboring intracranial tumors were examined daily for evidence of neurological symptoms, and their weights were obtained weekly. The animals were killed when they manifested hemiparesis or weight loss of at least 20% of their body weight. Brain specimens were examined histologically to confirm that the cause of death was tumor growth in each case. The animals harboring subcutaneous vaccine site tumors that were larger than 1.5 cm in maximal diameter were killed. Survival curves were compared using a log-rank test.

For the production of apoptotic glioma cells to be used in the in vivo studies described below, 9L cells were treated with 200 ng/ml UCN-01 in vitro for 72 hours in initial studies or 300 ng/ml UCN-01 for 96 hours in subsequent experiments. Control 9L cells were treated with vehicle alone. Cells were then harvested and washed before co-culture with DCs. Three general aims were pursued in the in vivo studies, as described below.

Assessment of the therapeutic activity of DCs co-cultured with apoptotic glioma cells

Our initial studies were designed to determine whether vaccination with DCs co-cultured with apoptotic 9L cells improved survival as compared with administration of apoptotic 9L cells alone or with DCs co-cultured with nonapoptotic (i.e., vehicle-treated) 9L cells, and whether these groups had improved survival as compared with animals that received unmodified 9L. On Day 0, rats (n = 24) underwent intracranial implantation of 1 × 10^4 9L cells. Immediately thereafter, the animals were treated with subcutaneous injections of the following: 1 × 10^4 9L cells (n = 6); UCN-01-treated (apoptotic) 9L cells (1 × 10^5) (n = 6); DCs (5 × 10^5) co-cultured with vehicle-treated (nonapoptotic) 9L (1 × 10^5) (n = 6); or DCs (5 × 10^5) co-cultured with UCN-01-treated 9L (1 × 10^5) (n = 6). The animals that received DCs co-cultured with vehicle-treated 9L or UCN-01-treated 9L underwent repeat subcutaneous injections with co-cultured cells 1 and 4 days after tumor implantation. At these time points, the other animals received additional injections of vehicle only.

Determination of whether co-culture is essential for therapeutic efficacy

On the basis of our initial results, we next questioned whether co-culture of DCs with apoptotic 9L cells was essential for prolonging survival or whether delivery of apoptotic 9L cells and DCs at separate sites would generate comparable results. On Day 0, the animals (n = 14) underwent intracranial implantation of 1 × 10^4 9L cells. Additional Day 0 treatment included the following: subcutaneous injection of HBSS (n = 4); subcutaneous injection of 1 × 10^6 DCs and 2 × 10^5 UCN-01-treated 9L cells at separate sites (n = 5); or subcutaneous injection of 1 × 10^6 DCs co-cultured with 2 × 10^5 UCN-01-treated 9L cells (n = 5). The injections were repeated 1 and 4 days after tumor implantation.

Evaluation of intensification of the vaccination schedule

Last, we examined whether intensification of the vaccination schedule could achieve long-term survival even in the setting of preestablished intracranial tumor. The animals (n = 32) underwent intracranial injection of 1 × 10^4 9L cells on Day 0. Treatment groups included the following: “control” animals (n = 8) that received subcutaneous injection of HBSS; 2 “DC-only” animals (n = 8) that received subcutaneous injection of 1 × 10^4 DCs; “separate injection” animals (n = 8) that received subcutaneous injections of 1 × 10^4 DCs and 2 × 10^5 UCN-01-treated 9L cells at separate sites; or “co-cultured” animals (n = 8) that underwent subcutaneous injection of 1 × 10^4 DCs co-cultured with 2 × 10^5 UCN-01-treated 9L cells. The treatment schedule for this experiment consisted of injections on Days 3, 4, and 7; Days 10, 11, and 14; Days 17, 18, 21; and Days 24, 25, and 28. For each series of injections (e.g., Days 3, 4, and 7), the respective cells were injected immediately (Days 3, 10, 17, and 24), incubated for 24 hours in vitro before injection (Days 4, 11, 18, and 25), or incubated for 96 hours in vitro before injection (Days 7, 14, 21, and 28). Four of six long-term (>90 d) survivors from the co-cultured vaccination group underwent rechallenge with intracranial injection of 1 × 10^4 9L cells in the opposite hemisphere.

RESULTS

Assay of physical interaction between DCs and UCN-01-treated apoptotic 9L glioma cells

Two-color flow cytometry of OX-6/fluorescein isothiocyanate (major histocompatibility complex class II)-labeled DCs and PKH26-labeled 9L cells is shown in Figure 1. Whereas immediate (<30 min) co-culture of UCN-01-treated 9L cells with DCs largely retained distinct populations of PKH26+/OX-6+ DCs and PKH26+OX-6- glioma cells, co-cultures of UCN-01-treated 9L cells that had been incubated for 24 hours with DCs demonstrated a sizable percentage of PKH26+/OX-6- cells. These results indicate that a physical association exists between apoptotic glioma cells and DCs after a period of incubation.
Assessment of vaccination strategies in an intracranial 9L gliosarcoma model

Assessment of the therapeutic activity of DCs co-cultured with apoptotic glioma cells

The initial studies were designed to assess the efficacy of vaccination with apoptotic 9L cells co-cultured with DCs in animals harboring intracranial tumors. As shown in Figure 2, a statistically significant improvement in survival was observed for animals that had been vaccinated with DCs co-cultured with UCN-01-treated (i.e., apoptotic) 9L cells relative to control groups that had been vaccinated with vehicle-treated (i.e., nonapoptotic) 9L cells alone, vehicle-treated 9L cells co-cultured with DCs, or UCN-01-treated 9L cells alone (\(P = 0.004, 0.004, 0.01\), respectively). However, no long-term (i.e., >90 d) survivors were observed in any of the groups. Not unexpectedly, the animals that had received vehicle-treated 9L cells (i.e., live, untreated tumor cells) developed enlarging tumors at the subcutaneous “vaccination” site. Small vaccine-site tumors also were noted in three of six animals immunized with DCs co-cultured with UCN-01-treated 9L and in all six animals immunized with UCN-01-treated 9L cells alone. Given that vaccine site tumors were observed in these animals, we increased the concentration of UCN-01 to 300 ng/ml and the incubation time to 96 hours in subsequent experiments.

Determination whether co-culture is essential for therapeutic efficacy

On the basis of the initial results, which demonstrated prolongation of survival in animals that received UCN-01-treated 9L cells co-cultured with DCs, we examined whether co-administration of DCs and apoptotic glioma cells was essential to provide an antitumor effect. Therefore, these studies included an additional control group of animals that received vaccinations with DCs and apoptotic 9L cells at separate sites. Survival curves are shown in Figure 3. The animals that received DCs co-cultured with UCN-01-treated 9L had significantly longer survival than either HBSS-treated controls or animals that received vaccinations with UCN-01-treated 9L cells and DCs at separate sites (\(P = 0.003\) and 0.04, respectively). A more modest but statistically significant improvement in survival relative to controls also was observed in animals that had received vaccination with DCs and UCN-01-treated 9L at separate injection sites (\(P = 0.03\)). There were

FIGURE 1. Two-color flow cytometry of OX-6/fluorescein isothiocyanate (major histocompatibility complex class II)-labeled DCs and PKH26-labeled UCN-01-treated 9L cells after immediate co-culture (A) and after incubation for 24 hours (B). After immediate co-culture, distinct populations of PKH26+/OX− glioma cells and PKH26−/OX-6+ DCs are noted, whereas after incubation for 24 hours, a large population of PKH26+/OX-6+ double-positive cells are noted.

FIGURE 2. Survival was significantly improved in animals vaccinated with 7-hydroxystaurosporine (UCN-01)-treated (apoptotic) 9L cells and dendritic cells (DCs) as compared with those vaccinated with vehicle-treated (nonapoptotic) 9L cells and DCs (\(P = 0.004\)), UCN-01-treated 9L cells alone (\(P = 0.01\)), or vehicle-treated 9L cells alone (\(P = 0.004\)).

FIGURE 3. Survival was significantly improved in animals vaccinated with 7-hydroxystaurosporine (UCN-01)-treated 9L cells co-cultured with dendritic cells (DCs) as compared with those vaccinated with injections of UCN-01-treated 9L cells and DCs at separate sites (\(P = 0.04\)) or Hanks’ balanced salt solution (HBSS) alone (\(P = 0.003\)).
no long-term survivors, and no subcutaneous tumors were observed at any of the vaccination sites.

**Evaluation of intensification of the vaccination schedule**

In an attempt to determine whether modification of the vaccination procedure could produce long-term survivors and possible cure of previously established intracranial 9L tumors, a more intensive, sequential vaccination strategy was used. This approach demonstrated that the animals that had received vaccination beginning on posttumor implantation Day 3 with DCs co-cultured with UCN-01-treated 9L had significantly longer survival times than did the HBSS-treated controls (P < 0.0001), the animals treated with DCs only (P < 0.0001), and the animals treated with injections of UCN-01-treated 9L cells and DCs at separate sites (P = 0.01). Long-term survival (>90 d) was observed in 6 (75%) of 8 co-culture-treated animals versus 1 (12.5%) of 8 animals that had received apoptotic 9L cells and DCs at separate sites, and 0 (0%) of 16 controls (Fig. 4). Three of four long-term survivors that were rechallenged with intracranial 9L in the opposite hemisphere also survived over the long term (>90 d).

With the sequential injection strategy, some animals developed subcutaneous tumors at the vaccine site. In animals that had received vaccination with DCs co-cultured with UCN-01-treated 9L, a small mass was observed at the vaccine site in five of eight cases. In each case, the maximum diameter did not exceed 0.5 cm, and complete regression was observed in all cases by Day 65. In contrast, the animals that had been vaccinated with separate injections of DCs and UCN-01-treated 9L developed a mass at the vaccine site that was confirmed to be tumor in all eight cases and that demonstrated steady growth in each case.

**DISCUSSION**

DCs are endogenous antigen-presenting cells that act as initiators and modulators of an immune response. Novel antitumor therapy in experimental models has recently focused on the use of “pulsed” DC vaccinations. In these models, DCs may be loaded in vitro with tumor-specific antigenic peptides, tumor cell extract or lysate, deoxyribonucleic acid- or ribonucleic acid-encoding tumor antigens, and apoptotic tumor cells (4, 8, 17, 19, 26). The rationale for the use of these methods of DC loading rather than simple co-culture with live tumor cells is that these cells generally are poorly immunogenic, unless they are genetically modified by transfection (1, 25, 27, 30, 38), possibly because they secrete a variety of immunologically suppressive substances such as Fas ligand and transforming growth factor β, which may impair DC activity and impede the efficacy of an induced immune response (29, 31, 33). In contrast, DCs pulsed with tumor antigens have been demonstrated to induce a potent antitumor T cell response in host animals (4, 19, 42). Furthermore, potent central nervous system immunity has been demonstrated with DCs pulsed with tumor antigens (26). However, the lack of universal tumor-specific antigens in human malignant gliomas is a limitation of this approach. Because a potent T cell response also has been reported with the use of apoptotic cell antigens that cross-presented by DCs (2, 3), we investigated the applicability of such an apoptotic tumor-based DC vaccination strategy as a therapeutic approach in a rat glioma model.

We applied a novel technique for the induction of tumor cell apoptosis using signal transduction inhibition. In preliminary studies, we demonstrated that UCN-01, a protein kinase C and cyclin-dependent kinase inhibitor, induced time-dependent apoptosis in glioma cell lines with a peak in the apoptotic index after 3 to 4 days of continuous drug exposure at a concentration of 200 to 300 ng/ml, depending on the cell line (6). Results from the current study using a terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end-labeling assay confirmed that tumor apoptosis was achieved in rat 9L gliosarcoma cells after incubation with 200 or 300 ng/ml of UCN-01 for 72 or 96 hours. Although a high percentage (>50%) of cells were rendered apoptotic with the lower-dose 3-day treatment regimen, complete tumor cell killing was not achieved, which is consistent with the observation that enlarging subcutaneous tumors were noted at the vaccination site in some of the animals that had received cells treated with this dose. To further diminish the percentage of viable cells, the concentration of UCN-01 was increased in subsequent experiments to 300 ng/ml, and the in vitro incubation period with 9L was increased to 96 hours. In these studies, enlarging vaccine site tumors did not develop in the animals that had received the apoptotic body-pulsed DC vaccine. Although some of these animals transiently developed a small (<5 mm) mass at the vaccine site, complete regression was noted in each case, suggesting that this manifestation reflected a local inflammatory response that eradicated any
remaining viable tumor cells or tumor cell components at the vaccine site. No subsequent tumor growth was observed during the entire follow-up period, which extended past 180 days. These results were confirmed in a subsequent cohort of animals, in which no enlarging vaccine site tumors were detected after apoptotic glioma cell-pulsed DC vaccination, despite more than 120 days of survival.

A recent report demonstrated that the efficacy of a DC-tumor cell vaccine in an experimental model for melanoma was dependent on a physical interaction between tumor cells and DCs (8). The results of the current study support this view. We have demonstrated through two-color flow cytometry that UCN-01-treated 9L cells and DCs form a physical interaction after in vitro culture for 24 hours. The majority of cells stained for both the PKH26-labeled component (9L cells) and the fluorescein isothiocyanate-labeled OX-6+ (DC) component. In contrast, UCN-01-treated 9L cells and DCs that were mixed immediately before flow cytometry exhibited separate populations of staining, indicating that the development of a physical interaction between tumor cells and DCs requires a period of co-culture. Although these flow cytometry data do not confirm the type of physical interaction between the apoptotic cells and the DCs (e.g., engulfment versus other physical association, such as attachment), confocal microscopic studies indicated that the interaction was one of engulfment of the apoptotic glioma cells by the DCs (data not shown). Presumably, these apoptotic glioma cell-pulsed DCs were able to process and present relevant antigens to the host immune system that not only prevented growth of remaining viable glioma cells at the subcutaneous vaccine site but also led to regression of a previously established tumor at a distant intracranial site.

A vaccination strategy using these glioma apoptotic body-pulsed DCs demonstrated substantial efficacy in a 9L rat intracranial tumor model. Although some prolongation of survival was observed after administration of apoptotic glioma cells that had not been co-cultured with DCs, this effect was significantly less potent than that observed with DCs that had been co-cultured with apoptotic glioma cells. The modest efficacy achieved with administration of apoptotic glioma cells alone presumably reflected some level of endogenous antigen uptake and presentation by DCs residing in the vaccine site microenvironment. These cells would perform the same role as the co-cultured DCs, although perhaps less efficiently, because our ex vivo co-culture approach permitted the isolation of large numbers of DCs that had been stimulated appropriately by cytokine administration before their priming with apoptotic glioma cells. Thus, animal survival in the apoptotic cell/DC co-culture group was significantly better after both single and multiple courses of vaccination than it was in the group that received co-cultured DCs and unmodified (i.e., vehicle-treated) glioma cells or the group that received apoptotic tumor cells alone. Six (75%) of eight animals exhibited long-term survival with the sequential vaccination strategy consisting of DCs co-injected with tumor cells that were induced to undergo apoptosis by UCN-01 pre-treatment. A memory response was also achieved in the majority of these long-term survivors, because three (75%) of four animals that were rechallenged by intracranial injection also survived over the long term. The exact antitumor mechanism of this vaccination approach is the focus of future investigations, but it is likely to be the result of a cell-mediated immune response, as has been demonstrated in other glioma vaccination models. Our previous studies, in which we used cytokine gene-transduced glioma cells as a vaccination strategy, demonstrated abundant infiltration of the primary tumor site by CD4+ and CD8+ lymphocytes in the setting of tumor rejection (22, 25–27, 41) and confirmed the presence of a tumor-specific immune response that could be transferred adoptively to induce an antitumor response in naive animals (12, 41).

Because of the efficacy and applicability of DC-based vaccination approaches, clinical implementation of apoptotic body-pulsed DCs as a vaccination strategy for high-grade gliomas has been developed as a Phase I clinical trial at our center. Investigators in other ongoing studies are examining which glioma-expressed antigens are responsible for mediating an effective antitumor immune response (24).

ACKNOWLEDGMENTS

TFW received support from the American College of Surgeons Resident Research Scholarship and the Copeland Fund of the Pittsburgh Foundation. HO receives support from the Competitive Medical Research Fund of the University of Pittsburgh Medical Center and the Pittsburgh Foundation’s Copeland Fund. WHC is the recipient of Grant CA68550 from the National Institutes of Health. IIFP is the recipient of Grants NS37704, NS01810, and CA81453 from the National Institutes of Health. The authors have no personal or financial interest in drugs, materials, or devices described in this article.

Received, July 10, 2001.
Accepted, January 30, 2002.
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REFERENCES

Glioma Apoptotic Body-pulsed Dendritic Cell Vaccine


Neurosurgery, Vol. 50, No. 6, June 2002
COMMENTS

The authors have demonstrated that induction of apoptosis in glioma cells seems to increase the effectiveness of pulsing dendritic cells (DCs). Overall, this model is not very stringent, because 9L is immunogenic and the animals were treated on Day 0 rather than after established tumors were present. Numerous vaccinations were needed during the course of 28 days to cure these animals. At this frequency level, even the apoptotic 9L cells had an effect. Regardless of this observation, these findings are interesting and presented well. This information will be useful in clinical trials.

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DCs are powerful immunological adjuvants that are already finding application in tumor immunotherapy. In this report of a study using the 9L glioma model in Fischer rats, Witham et al. describe an effective vaccine consisting of DCs generated from bone marrow precursors using granulocyte-macrophage colony-stimulating factor, interleukin-4, and flt-3 ligand. The DCs are co-cultured with apoptotic 9L glioma cells. Animals that received three DC vaccinations immediately after intracranial 9L tumor implantation showed a modest but significant increase in survival time. An intensive DC vaccination schedule (12 vaccinations starting 3 d after 9L implantation) resulted in some long survival times.

These encouraging results are probably the result of DCs’ phagocytosing apoptotic bodies and cross-presenting tumor-associated antigens to T cells, which were then able to transfer to the brain and mediate the antitumor effects, although such mechanisms were not demonstrated directly in this study. In indirect support of immunological memory induction, however, three of four rats that demonstrated long-term survival were able to withstand a rechallenge of 9L glioma cells in the contralateral hemisphere.

Of particular interest with regard to this approach is that it does not necessitate prior identification of tumor antigens. However, this is also the case with regard to alternative DC-based vaccines that are charged with antigen by pulsing with tumor cell lysates or eluted peptides or by transfection with tumor cell deoxyribonucleic acid or ribonucleic acid. To compare these procedures and rationally select the best one for clinical trials, detailed information about the maturational, and thus the functional, status of the DC is needed. The authors undoubtedly will address this issue in the rat model they describe in this article, but it will be equally important with regard to the DCs eventually cultured for clinical application. Indeed, it should be borne in mind that DCs are multifaceted tools that are able to induce not only antitumor immunity but also immune tolerance and autoimmunity. Thus, immune response monitoring and a careful assessment of the impact of these immunotherapies on normal brain tissue will be prudent when developing these promising approaches for the clinic.

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With the advent of molecular biological and gene therapy techniques, there has been a considerable renewal of interest in investigating the potential of immunotherapy in the treatment of patients with malignant brain tumors. One of the reasons for the failure of previous immunotherapy trials was the limitation imposed by the brain tumor itself in its failure to present antigens to the immune system effectively and thus stimulate a potent immune response. A second limitation was the physician’s inability to deliver immunotherapy to the brain tumor effectively and sustain an immune response in the central nervous system. DCs have recently emerged as perhaps the immunologist’s “savior.” These cells are known to be potent antigen-presenting cells and thus may stimulate a sufficient immune response to be valuable in the treatment of intracerebral tumors.

The goal of the present study was to find a way to augment the immune response against a glioma by using stimulated DCs. The hypothesis of the study is that introducing apoptosis in a glioma might result in a stronger antitumor immune response by the DCs when the DCs are preincubated with apoptotic glioma cells before their use in a vaccine. This theory is derived from evidence suggesting that apoptotic cells provide a readily available source of tumor antigens for uptake, processing, and presentation by the DCs.
In this study, the authors explored the efficacy of subcutaneous vaccination with glioma apoptotic body-pulsed DCs in inhibiting intracerebral tumor growth in a syngeneic 9L glioma Fischer rat model. The results demonstrate that pretreatment of 9L cells with 7-hydroxystaurosporine, a stimulator of apoptosis, causes the development of apoptosis in 50% of the tumor cells as compared with only 3% of the controls. Furthermore, in animals harboring intracranial tumors, survival times were significantly longer for animals treated with a glioma apoptotic body-pulsed DC vaccine than they were in animals that received various control treatments. Surprisingly, no survival benefit was observed in animals that received DCs co-cultured with nonapoptotic 9L cells, which contradicts the results in some other studies reported in the literature. No explanation is offered for this finding. Two other problems with this article are that no systemic immune studies are reported and no histological confirmation is shown. The demonstration of immune memory in surviving animals rechallenged with tumor supports the role of the immune system, but further quantitative studies, especially in clinical trials, will be important. Another consideration for future investigation is whether other routes of delivery of DCs might be more effective. Nevertheless, this article provides more data to support the potential benefit of DCs in stimulating an antitumor response against an intracerebral tumor and their use in tumor vaccines.

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