Systemic T cell adoptive immunotherapy of malignant gliomas

GREGORY E. PLAUTZ, M.D., GENE H. BARNETT, M.D., DAVID W. MILLER, M.D.,
BRUCE H. COHEN, M.D., RICHARD A. PRAYSON, M.D., JOHN C. KRAUSS, M.D.,
MARK LUCIANO, M.D., PH.D., DEBRA B. KANGISSE, P.A.C., AND SUYU SHU, PH.D.

Departments of Neurological Surgery, Neurology, and Pathology, and the Center for Surgery Research,
The Cleveland Clinic Foundation, Cleveland Ohio

Object. To determine the feasibility, toxicity, and potential therapeutic benefits of systemic adoptive immunotherapy, 10 patients with progressive primary or recurrent malignant glioma received this treatment. Adoptive immunotherapy, the transfer of immune T lymphocytes, is capable of mediating the regression of experimental brain tumors in animal models. In animal models, lymph nodes (LNs) that drain the tumor vaccine site are a rich source of tumor-immune T cells.

Methods. In this clinical study, patients were inoculated intradermally with irradiated autologous tumor cells and granulocyte macrophage–colony stimulating factor as an adjuvant. Cells from draining inguinal LNs, surgically resected 7 days after vaccination, were stimulated sequentially with staphylococcal enterotoxin A and anti-CD3, and a low dose of interleukin-2 (60 IU/ml) was used to expand the stimulated cells. The maximum cell proliferation was 350-fold over 10 days of culture. The activated cells were virtually all T cells consisting of various proportions of CD4 and CD8 cells. These cells were given to patients by intravenous infusion at doses ranging from $9 \times 10^8$ to $1.5 \times 10^{11}$. There were no Grade 3 or 4 toxicities associated with the treatment. Following T-cell transfer therapy, radiographic regression that lasted at least 6 months was demonstrated in two patients with recurrent tumors. One patient demonstrated stable disease that has lasted for more than 17 months. The remaining patients had progressive disease; however, four of the eight patients with recurrent tumor remain alive more than 1 year after surgery for recurrence. Three patients required intervention with corticosteroid agents or additional surgery approximately 1 month following cell transfer.

Conclusions. These intriguing clinical observations warrant further trials to determine whether this approach can provide therapeutic benefits and improve survival.

Key Words • adoptive immunotherapy • T lymphocyte • malignant glioma • clinical trial • staphylococcal enterotoxin A

Gliomas are the most common primary tumor of the central nervous system in adults, accounting for approximately 50% of brain tumors. Malignant gliomas, including anaplastic astrocytoma and glioblastoma multiforme (GBM), occur in four to six new cases per 100,000 people per year. Aggressive multimodality therapy, including surgical resection, radiotherapy, and chemotherapy, were documented to play a beneficial role nearly 20 years ago. Unfortunately, despite current therapy, recurrence is usually rapid for GBM, with the 2-year survival rate remaining at approximately 5%. This difficult clinical situation has stimulated interest in additional approaches to the treatment of malignant gliomas. The use of tumor-reactive T lymphocytes for cancer therapy has particular theoretical appeal as an adjunct to current therapies.

In several clinical trials, the adoptive transfer of tumor-reactive T cells has been shown to mediate the regression of established tumors in a small number of patients with extracranial tumors. The quality of the T cells is probably the most important component of adoptive immunotherapy, and the source, specificity, and number of T cells are essential determinants of efficacy. Using preclinical animal tumor models, we have determined that lymph nodes (LNs) draining a tumor inoculum are the optimum source of T cells sensitized to specific tumor antigens, in contrast to other lymph tissues. After ex vivo stimulation and expansion, these cells developed into potent therapeutic effector cells which, on systemic transfer, were capable of mediating the regression of tumors established in the lung and skin as well as in the brain. The reactivity of the tumor-draining LN T cells was exquisitely specific for the tumor that provided the in vivo stimulation. Independently derived tumors of similar histological composition were not affected, suggesting that tumor-specific rather than tissue-restricted antigens were the dominant antigens in this immune response. Although similar tumor-reactive T cells may exist in can-
Adoptive immunotherapy of malignant glioma

Cancer patients, there is evidence that their ability to proliferate and function is suppressed.1,16 Most of the clinical applications of adoptive immunotherapy have been for melanoma and renal cell carcinoma. There are theoretical reasons to suggest that the application of T-cell immunotherapy delivered to malignant brain tumors might be difficult. First, the brain is considered an immune-privileged site, and diminished immune responses are generated against antigens introduced into the central nervous system.19,31 Second, it is well documented that many gliomas release substances such as transforming growth factor–β, prostaglandin E2, and interleukin (IL)-10, that cause immunosuppression.8,15,20,21,30 Third, because of the requirement for structural integrity and confined anatomical space, the brain may not tolerate the inflammation associated with an immune reaction. Despite these concerns, we have demonstrated in animal models that adoptive transfer of activated tumor-draining LN T cells mediated the regression of experimentally established intracranial malignancies.22,45,47 Transferred intravenously, T cells infiltrated intracranial tumors, demonstrating that there is no intrinsic barrier to the migration of activated T cells into intracranial tumors. The T-cell infiltrate was localized to the tumor; very few T cells were present in the normal brain parenchyma.34 A murine glioma was also successfully treated by adoptive transfer of activated tumor-draining LN T cells, and cured mice were neurologically normal and resisted a second intracranial tumor challenge.38 These data indicated that adoptive immunotherapy might be an alternative treatment for humans with malignant glioma. A phase I clinical trial was initiated to establish whether adoptive immunotherapy was feasible in patients with recurrent or residual malignant astrocytoma and to determine the toxicity associated with this treatment.

Clinical Material and Methods

Protocol Inclusion Criteria

Eligible patients had pathologically confirmed Grade III or IV malignant astrocytoma. All patients received radiotherapy and, in some cases, chemotherapy following their primary diagnosis. Pathological analysis confirmed recurrent disease in eight patients, and clear radiographic evidence of residual/recurrent tumor following radiotherapy was demonstrated in the two patients with primary GBM. The study was approved by the institutional review board, and written informed consent was obtained from all patients. Tissue was obtained at the time of medically necessary surgical debulking or resection. Eligibility criteria included a performance status of 0 to 1 on the Eastern Cooperative Oncology Group scale; clinical stability without concurrent corticosteroid treatment in excess of physiological replacement at the time of vaccination or lymphocyte infusion; white blood cell count greater than 2 × 10^9/µl, platelet count greater than 100 × 10^9/µl, blood urea nitrogen level less than 25 mg/dl, creatinine level less than 1.8 mg/dl, serum glutamic oxaloacetic transaminase level less than two times normal upper limit, and total bilirubin level less than 3 mg/dl; and negative serology for hepatitis B virus and human immunodeficiency virus.

No patient received chemotherapy, radiotherapy, or immunotherapy in the 4 weeks preceding vaccination. Pregnant or lactating women were excluded, as were patients with radiation implants, active or unexplained febrile illness, active collagen vascular disease, or who were otherwise immunologically compromised due to chronic conditions.

Vaccination Procedures

Fresh tumor specimens obtained at the time of tumor resection were transported at 4°C in sterile Hanks’ balanced salt solution (HBSS) to a dedicated tissue culture hood. Necrotic tumor, connective tissue, and blood clots were removed, and the tissue was minced with a scalpel. Single-cell suspensions were prepared by digestion in 40 ml of HBSS containing 4 mg DNase I, 40 mg collagenase type IV, and 100 U hyaluronidase type V at room temperature for 3 hours. Cells were filtered through a layer of No. 100 nylon mesh and washed twice in HBSS. Tumor cultures were initiated by adding cells to fibronectin-coated tissue culture flasks and cultured in Dulbecco’s modified Eagle’s medium (84%), supplemented with X-VIVO 15 (10%), human AB serum (5%), G5 (1%), and hydrocortisone (10 µg/ml) at 37°C, 5% CO2 in a humidified incubator. Routinely, short-term culture of tumor cells has been successful in approximately 70% of cases. Either cultured or fresh-frozen tumor cells were used for inoculation of patients. Tumor cells were irradiated (25 Gy) using a 137Cs source, centrifuged, and a range of 17 to 56 × 10^6 tumor cells were resuspended in 0.6 ml phosphate-buffered saline containing 250 µg granulocyte macrophage–colony stimulating factor (GM-CSF) (Sargramostim). The patients were injected intradermally on the anterior upper thigh bilaterally, and GM-CSF (125 µg) was injected intradermally into each inoculation site daily for an additional 3 days.

Activation of T Cells

Inguinal LNs draining the vaccine site were surgically removed 7 days after vaccination. Lymph nodes were minced into 1- to 2-mm fragments, teased apart with 20-gauge needles, and filtered through nylon mesh to obtain a single-cell suspension. The LN cells were activated in X-VIVO 15 containing 10% AB serum and 100 ng/ml staphylococcal enterotoxin A (SEA), at a density of 4 × 10^6 cells/well in 24-well tissue culture plates, at 37°C, 5% CO2 in a humidified incubator. Two days later, cells were centrifuged, washed, and resuspended at 10^7/ml in X-VIVO 15 supplemented with IL-2 (60 IU/ml). Five to 8 days after initiation of T-cell cultures, cells were restimulated for 14 hours by incubation in tissue culture flasks coated with anti-CD3 monoclonal antibody (mAb), OKT3. Cells were subsequently adjusted to a concentration of 10^7/ml and cultured in X-VIVO 15 with IL-2 (60 IU/ml) for an additional 5 to 10 days. Aliquots of cells were counted during the culture to determine the growth rate. Cultures were harvested using a modified blood cell separator and were suspended in 250 to 500 ml of 0.9% NaCl containing 5% dextrose, 1.25% human albumin, and IL-2 (60 IU/ml).

Adoptive Cell Transfer

Two days prior to cell infusion, patients received a sin-
Summary of clinical characteristics and treatment stages in 10 patients with malignant glioma*

<table>
<thead>
<tr>
<th>Case No.</th>
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<th>Initial Treatment</th>
<th>Date of Surgery for T-Cell Recurrence</th>
<th>Infusion</th>
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<td>GBM</td>
<td>XRT</td>
<td>NA</td>
<td>5/16/96</td>
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<td>54, F</td>
<td>12/95</td>
<td>GBM</td>
<td>BCNU, XRT</td>
<td>5/16/96</td>
<td>7/05/96</td>
</tr>
<tr>
<td>3</td>
<td>58, M</td>
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<td>AA</td>
<td>XRT</td>
<td>4/2/96</td>
<td>8/10/96</td>
</tr>
<tr>
<td>4†</td>
<td>16, F</td>
<td>7/89</td>
<td>GBM</td>
<td>XRT, gamma knife</td>
<td>8/14/96</td>
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</tr>
<tr>
<td>5</td>
<td>49, M</td>
<td>2/96</td>
<td>GBM</td>
<td>XRT</td>
<td>7/16/96</td>
<td>11/06/96</td>
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<td>6</td>
<td>71, F</td>
<td>7/96</td>
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<td>XRT</td>
<td>NA</td>
<td>11/14/96</td>
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<tr>
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<td>42, M</td>
<td>1/96</td>
<td>GBM</td>
<td>radiosurgery, DTI-015</td>
<td>7/5/96</td>
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</tr>
<tr>
<td>8‡</td>
<td>51, M</td>
<td>10/95</td>
<td>GBM</td>
<td>XRT, PCV</td>
<td>8/2/96</td>
<td>11/27/96</td>
</tr>
<tr>
<td>9</td>
<td>20, M</td>
<td>5/96</td>
<td>GBM</td>
<td>XRT</td>
<td>9/13/96</td>
<td>12/02/96</td>
</tr>
<tr>
<td>10</td>
<td>48, F</td>
<td>12/95</td>
<td>GBM</td>
<td>BCNU, CDDP, XRT</td>
<td>9/10/96</td>
<td>12/03/96</td>
</tr>
</tbody>
</table>

* CDDP = cisplatin; DTI-015 = carmustine in ethanol for intralesional use; NA = not applicable; PCV = procarbazine, lomustine, vincristine; XRT = external beam radiotherapy.
† Initially diagnosed as gangliocytoma; recurrent diagnosis of GBM.
‡ Initially diagnosed as gemistocytic astrocytoma; recurrent diagnosis of GBM.

Toxicity and Response Criteria

Toxicity was monitored according to National Cancer Institute Common Toxicity Criteria. Complete response was defined as disappearance of all clinical and radiographic evidence of tumor; partial response as at least a 50% decrease in the sum of the cross-sectional areas of tumors; stable disease was defined as any change too small to quantify as either partial response or progressive disease; and progressive disease was defined as the appearance of a new lesion or an increase in the cross-sectional area of a measured lesion by more than 25%.

Flow Cytometry

Cell suspensions obtained from LNs or after ex vivo stimulation were stained directly with conjugated mAb directed against CD3, CD4, CD8, CD25, and HLA-DR. Data from 10,000 stained cells were collected and analyzed on a flow microfluorometer equipped with CellQuest software.

Sources of Supplies and Equipment

The Sargramostim was kindly provided by Immunex Corp., Seattle, WA. The SEA, the human AB serum, and the enzymes used in the single-cell suspensions were obtained from Sigma Chemical Co., St. Louis, MO. The Dulbecco’s modified Eagle’s medium and G5 were acquired from Life Cell Technologies, Grand Island, NY.

The anti-CD3 mAb OKT3 was obtained from Ortho Biotech, Raritan, NJ; the X-VIVO from Biowhittaker Inc., Walkersville, MD; the IL-2 from Chiron Therapeutics, Emeryville, CA; the DTI-015 from Direct Therapeutics Inc., White Plains, NY; and the stains used in flow cytometry from Becton Dickinson, Sunnyvale, CA.

The nylon mesh (Nytex) was purchased from Tetko Inc., Briarcliff Manor, NY. The blood cell separator (CS-3000) used to harvest cultures was acquired from Baxter, Deerfield IL. The software (CellQuest) and flow cytometer (FACScan) were obtained from Becton Dickinson.

Results

Patient Characteristics

Ten of 11 consecutively presenting eligible patients were treated by T-cell adoptive transfer; their characteristics are summarized in Table 1. The patient in Case 3 had recurrent Grade III astrocytoma, the patients in Cases 1 and 6 had persistent GBM following standard external-beam radiotherapy, and the other seven patients had recurrent GBM. All patients were treated with conventional radiotherapy, four of whom also received chemotherapy following their primary diagnosis. The time between surgical removal of tumor to obtain cells for the vaccine and cell infusion was typically between 10 and 14 weeks but varied between 7 weeks for Case 2 and 6 months for Case 1. Prior to vaccination, magnetic resonance (MR) imaging was used to document residual/recurrent tumor following the completion of radiotherapy in two patients (Cases 1 and 6) in whom tumor was obtained at initial diagnosis. Postoperative MR imaging demonstrated residual tumor in eight patients with recurrent tumors and in six, tumor growth was clearly apparent in the interval between surgery for recurrence and T-cell infusion.

Cultured Tumor Cell Vaccination

Cultured tumor cells were used in nine patients, although in two patients (Cases 2 and 3) the cultured cells were supplemented with fresh-frozen tumor to bring the total cell dose to greater than 20 × 10⁶. Cultured tumor cells were not available for one patient (Case 7) and fresh-frozen tumor cells were used for vaccination. Four patients (Cases 4, 5, 8, and 10) had undetectable or minimal inguinal LN enlargement following the first vaccination, and vaccination was repeated 2 weeks later; each of these patients developed a response to the second vaccination. Typically, the vaccination site had 5 to 8 mm of induration and a halo of erythema up to 5 cm in diameter. Mild tender-ness and pruritus at the vaccine site occurred in several patients. The erythema subsided within 3 days after completion of the GM-CSF injections. There was no evidence of growth of tumor cells at the vaccine site in any patient through the duration of follow up.

Growth of LN T Cells

The inguinal LNs draining the vaccine site were surgically removed 7 days after vaccination and processed to a single-cell suspension. The number of cells obtained from the vaccine-draining LNs and their ex vivo growth are described in Table 2. The number of cells obtained from

<table>
<thead>
<tr>
<th>Patient Characteristics</th>
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</thead>
<tbody>
<tr>
<td>Age Date of Initial Date of Date of Treatment</td>
</tr>
<tr>
<td>Case (yrs)</td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
<td>1</td>
</tr>
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<td>2</td>
</tr>
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<td>3</td>
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<td>9</td>
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</table>

* CDDP = cisplatin; DTI-015 = carmustine in ethanol for intralesional use; NA = not applicable; PCV = procarbazine, lomustine, vincristine; XRT = external beam radiotherapy.
† Initially diagnosed as gangliocytoma; recurrent diagnosis of GBM.
‡ Initially diagnosed as gemistocytic astrocytoma; recurrent diagnosis of GBM.
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The LNs varied from 1.1 \times 10^4 to 5.6 \times 10^6 (average 2.6 \times 10^6). The cells were activated with SEA for 2 days, and during this initial activation, the cells formed large aggregates and morphologically became lymphoblasts. The cells were washed and resuspended at a low density (10^4/ml) in serum-free media containing IL-2. The cells proliferated between seven- and 27-fold following SEA activation. Five to 8 days later, the cells were exposed to immobilized anti-CD3 mAb, and they expanded in IL-2 for an additional 5 to 10 days. Proliferation of T cells varied from 46 to 89% with the remainder consisting mostly of B lymphocytes and monocytes. In most cases, the majority of the LN T cells were initially CD4 cells; however, in Cases 5 and 6, CD8 cells were predominant. A component of the IL-2 receptor, CD25 is expressed on the cultured T cells was highly variable. The expression of CD25 on the cultured T cells was highly variable.

**TABLE 2**
Summary of responses to vaccination, T-cell activation, and cell transfer*

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Tumor Cells</th>
<th>LN Cells</th>
<th>Fold Increase</th>
<th>No. of Cells Infused</th>
<th>Response (duration)</th>
<th>Survival (mos)</th>
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<tr>
<td>1</td>
<td>C</td>
<td>5.6</td>
<td>10</td>
<td>350</td>
<td>1.5 \times 10^4</td>
<td>PD</td>
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<tr>
<td>2</td>
<td>C + F</td>
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<td>58</td>
<td>1.7 \times 10^4</td>
<td>PD</td>
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<td>PR (6 mos)</td>
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<tr>
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<td>C</td>
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<td>PD</td>
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<tr>
<td>5</td>
<td>C</td>
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<tr>
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<tr>
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<td>F</td>
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<td>229</td>
<td>6.0 \times 10^4</td>
<td>PR (&gt;13 mos)</td>
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<tr>
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<td>PR (7 mos)</td>
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<td>154</td>
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</tr>
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</table>

* PD = progressive disease; PR = partial response.
† Patient received cultured tumor cells.
‡ Patient received frozen tumor digest.
§ Time from resection of tumor.
|| Time from resection of recurrent tumor.
** Patient was removed from study 4 months after cell transfer for additional therapy.

**TABLE 3**
Characteristics of vaccine-draining LN cells*

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<tr>
<th>Case No.</th>
<th>T Cells</th>
<th>CD4</th>
<th>CD8</th>
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<td>ND</td>
<td>100</td>
<td>71</td>
<td>25</td>
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</tr>
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</table>

* ND = not done.

**Clinical Outcome**

All patients treated by using this protocol had residual or recurrent tumors, and clear radiographic tumor progression was demonstrated in nine patients between the time of surgery and T-cell transfer. Despite active disease and rapid tumor progression prior to initiation of immunotherapy, three patients demonstrated tumor responses of varying degrees following immunotherapy. Stereotactic biopsy performed in November 1995 was used to diagnose low-grade astrocytoma of the right frontal lobe in the patient in Case 3 but radiographic evidence showed recurrence in February 1996, 3 months after diagnosis and local radiotherapy. A nearly complete surgical resection of the recurrent tumor that was performed 2 months later revealed a Grade III astrocytoma; however, significant tumor growth occurred in the 3-month interval between the last surgery and initiation of immunotherapy, during which time no additional therapy was administered. Comparison of pretreatment to follow-up MR images showed transient tumor regression that lasted for 6 months after immunotherapy. Interestingly, the patient was taking dexamethasone at the time of the last two MR studies (Fig. 1E and F). This patient received subsequent treatment with tamoxifen and survived 20 months after the surgery for recurrence.
The patient in Case 7, in whom a GBM was diagnosed in January 1996, was initially treated with surgery, conventional radiotherapy, and stereotactic radiosurgery. Three months later the tumor recurred, and the patient was treated on May 1, 1996, with intratumoral instillation of carmustine (BCNU) in absolute ethanol, DTI-015. Subsequent MR studies showed progressive disease and the patient underwent repeated surgery on July 30, 1996. As shown in Fig. 2, minimal disease was apparent on postoperative MR images following surgery for recurrent tumor; however, interval progression occurred in the absence of additional therapy, prior to immunotherapy, which began on November 18, 1996. Subsequent imaging studies at 1, 3, 5, and 7.5 months after T-cell transfer demonstrated stable disease, with some fluctuation in levels of enhancement at the margins of tumor resection site. Clinically, more than 17 months after surgery for recurrence, this patient has remained stable and has not required corticosteroid treatment.

The patient in Case 9 underwent repeated resection of a recurrent GBM on September 13, 1996, 4 months after initial diagnosis and treatment with external-beam radiotherapy. No additional treatment was administered between surgery and immunotherapy, which began on November 18, 1996. Subsequent imaging studies at 1, 3, 5, and 7.5 months after T-cell transfer demonstrated stable disease, with some fluctuation in levels of enhancement at the margins of tumor resection site. Clinically, more than 17 months after surgery for recurrence, this patient has remained stable and has not required corticosteroid treatment.

In seven patients the tumors increased in size between the date of T-cell transfer and the first follow-up MR image obtained within 2 months. This was associated in six patients with progression of clinical symptoms requiring the use of dexamethasone. Two patients (Cases 1 and 2) were treated with surgical debulking because of an increase in clinical symptoms approximately 4 weeks after cell transfer. These two patients subsequently experienced progressive tumor growth and died 6 months after initiation of immunotherapy. The patient in Case 10 developed tumor progression and was removed from the study 4 months after cell transfer for surgical debulking and placement of a Gliadel wafer. The patient in Case 5 exhibited rapid tumor growth (3.5 × 4.5 cm) in the 3-month interval between subtotal resection of recurrent tumor and T-cell transfer. Although subsequent imaging has demonstrated a slow increase in the size of tumor and/or edema, he survived 9 months after immunotherapy and 13 months.

![Fig. 1. Case 3. Serial MR images obtained following surgery for tumor recurrence (A), prior to immunotherapy (B), and following T-cell transfer, at 1 month (C), 2 months (D), 4 months (E), and 6 months (F).](image-url)
after surgery for recurrence, suggesting that the rate of tumor growth was attenuated.

Discussion

The results of this study demonstrate the feasibility and lack of toxicity of adoptive immunotherapy for the treatment of malignant gliomas. The study design is based on overwhelming preclinical data documenting that LNs draining a progressive tumor or tumor vaccine are a rich source of tumor-sensitized T cells. A key feature of this clinical protocol is the use of autologous tumor cells to stimulate T lymphocytes in the draining LN. The antigens from malignant gliomas that are recognized by T lymphocytes have not yet been characterized, and it is not known if they are unique to each tumor or are shared. However, we reasoned that short-term cultured autologous tumor cells would likely be an adequate source of such antigens. The cultured tumor cells were free of necrotic cells, debris, and other types of cells; thus, they provided a more uniform vaccine preparation than frozen cells from surgical specimens. Moreover, it has been demonstrated in a previous study that tumor cells retain their antigenicity following short-term culture.

Because it promotes the development and activation of antigen-presenting cells, GM-CSF was chosen as an adjuvant for the tumor cell vaccine. It is currently under investigation as an adjuvant for many clinical applications and has demonstrated great promise in promoting tumor immune responses. In patients with malignant glioma, GM-CSF induced local erythema at the vaccine site; however, in contrast to bacillus Calmette–Guérin vaccine, which has been used in previous immunotherapy protocols, no local ulceration or infection was observed. Additionally, tumor growth was never observed at the vaccination site, possibly because of irradiation of the vaccine and the poor metastatic capacity of gliomas. Thus, vaccination with autologous tumor and GM-CSF was feasible and well tolerated.

A number of investigations have established that there is a generalized suppression of the immune system in patients with malignant gliomas. Initially, in our study, it was uncertain whether vaccination would induce a response in the draining LN for these patients. Indeed, four patients with large tumors required a second vaccination to stimulate a hyperplastic LN response. The yield of cells from the draining LN in these four patients averaged $1.3 \times 10^8$ cells, whereas the average cell yield from the remaining six patients was $3.6 \times 10^8$ cells. Even in the patients with a more favorable response to the vaccine, the yield of cells is depressed when compared with patients with renal cell carcinoma or melanoma receiving vaccination in similar clinical protocols. Despite the existence of immunosuppression in vivo, the LN T cells responded...
well to ex vivo activation. A significant advantage of the ex vivo activation of T cells with SEA and anti-CD3 mAb is that vigorous proliferation occurred even in a low concentration of IL-2 (60 IU/ml). Biologically, SEA is probably the most potent stimulus for human T cells. Although considered a superantigen, SEA is capable of activating the majority of T cells, thus acting as a mitogen. In five patients treated on this protocol, there was a greater than 100-fold proliferation of cells. Because the therapeutic efficacy of adoptive immunotherapy is proportional to the number of transferred cells, we hypothesize that, in addition to activating T cells, the numerical expansion of T cells that occurs during ex vivo culture augments the immunotherapy. The ex vivo activation with SEA and anti-CD3 mAb and IL-2 stimulated both CD4 and CD8 cell proliferation. In all cases, the percentage of CD8 cells increased during the culture, and in several cases CD8 cells were predominant at the time of infusion into patients. It has not yet been determined whether both CD4 and CD8 T cells are required to mediate the regression of human gliomas. Results from our experimental animal models support the hypothesis that both CD4 and CD8 T cells are required to achieve regression of intracranial tumors.

To mediate tumor regression, the systemically transferred cells must migrate to the tumor and retain the capacity to respond to tumor antigens. In animal experiments, we have determined that despite the nonspecific nature of the T-cell activation induced by SEA or anti-CD3, the cells retained their antigen specificity. The use of low concentrations of IL-2 during activation is extremely important because the T cells lose their antigen specificity when cultured in high concentrations of IL-2 (for example, 600 IU/ml). Adoptive immunotherapy has been used to treat many types of malignancies: for example, lymphokine-activated killer (LAK) cells generated from peripheral blood leukocytes by culture in high doses of IL-2 (6000 IU/ml) and maintained in vivo with high doses of systemic IL-2 following transfer. Unfortunately, clinical trials with LAK cells failed to show benefits above those observed with IL-2 only, possibly because of their poor migration to the tumor site.

To overcome the problem of migration to malignant brain tumors, intracavitary infusion of LAK cells or allogeneic CTL cytotoxic T lymphocytes and IL-2 has been used in a number of clinical trials. However, the results of these studies have been uniformly disappointing. Apparently, the mere presence of activated T cells in the tumor cavity is not sufficient to mediate tumor regression. The systemic delivery of T cells in the current protocol, in contrast with intracavitary delivery, does not pose a significant problem, provided the cells retain their ability to home to sites of antigen. In addition, because gliomas infiltrate surrounding normal brain tissue, many tumor cells may actually be physically closer to brain capillaries than the surface of...
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the tumor cavity. It is possible that limited diffusion of T cells from the tumor cavity is one factor that may have limited the efficacy of intracavitary adoptive immunotherapy in previous clinical trials.

In nearly all of the previous studies of adoptive cellular immunotherapy, systemic IL-2 has been administered to patients as a conjunctural treatment. In fact, most of the toxicities associated with these treatments, particularly neurological toxicity, are attributed to IL-2. Studies in animals with intracranial tumors have revealed that systemic IL-2 actually inhibits the efficacy of adoptive immunotherapy. The detrimental effect of IL-2 for the treatment of intracranial tumors stands in marked contrast to its beneficial effect against tumors in other visceral organs. This unique feature of the use of systemic IL-2 in conjunction with adoptive immunotherapy possibly interfered with the therapeutic effect against brain tumors in previous clinical trials. In the current study, systemic IL-2 was not administered to the patients. Although it is not known with certainty whether IL-2 interferes with T-cell migration to brain tumors in humans, the deletion of systemic IL-2 has contributed to the low toxicity exhibited in this treatment protocol.

The toxicity associated with vaccination, inguinal LN resection, and T-cell infusion was minimal, and these procedures were all performed in an outpatient setting. Tumor enlargement and increased cerebral edema occurred approximately 1 month after cell transfer in three patients and could possibly represent a form of delayed toxicity. Each of these patients had significant tumor burden prior to treatment, and in two cases tumor progression was documented on pathological examination 1 month after cell transfer. In these cases it is not known whether the exacerbation of clinical symptoms and tumor enlargement observed on MR imaging represented the natural tumor progression, an accelerated progression of tumor growth, or edema secondary to inflammation induced by the cell transfer. Cytokines produced by T lymphocytes at sites of inflammation could contribute to vasogenic edema in the absence of glucocorticoid use. It is notable, however, that two patients (Cases 7 and 9) with radiographic and clinical responses did not develop symptoms of cerebral edema during tumor regression. It is likely that the size of the tumor and the surrounding brain’s capacity to accommodate swelling would contribute to the risk of toxicity caused by inflammation and edema. These findings suggest that patients with large tumor masses or tumor located in critical brain structures may not be preferred candidates for adoptive immunotherapy.

Despite the theoretical concern regarding the development of immune reactions to shared antigens on normal brain tissue, we did not find radiographic or clinical evidence to support this possibility in any of the patients. In instances in which there was radiographic evidence of cerebral edema, it was confined to the tumor and adjacent brain. Because of the rapid progression of recurrent malignant gliomas in most of these patients, we have not yet been able to determine whether delayed autoimmune reactions against normal brain will develop as a late complication. However, unlike radiotherapy or chemotherapy in which certain side effects are unavoidable aspects of therapy, immunotherapy achieves clinical responses with minimal side effects.

The current protocol was designed to test whether adoptive immunotherapy with autologous LN T cells was feasible and to determine the associated toxicities. Because it is necessary for patients to be clinically stable and not receiving corticosteroids, there may be brain tumor patients dependent on corticosteroids to control symptoms for whom this approach would not be possible. The general protocol was designed to test treatment efficacy relative to controls or another therapy. However, the survival of these 10 patients treated with adoptive immunotherapy compares favorably with the 23-week median survival observed for surgery alone in patients with recurrent gliomas in a recent large clinical trial. Moreover, the clinical and radiographic responses observed in three patients with recurrent malignant gliomas are very encouraging, given the tumor burden at the initiation of the treatment. Therefore, further study of adoptive immunotherapy immediately following standard surgery and radiation therapy for primary malignant glioma is warranted to determine therapeutic efficacy and survival benefits.

Disclosure

The authors do not have a financial interest in the methodology described in this manuscript.

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Address reprint requests to: Suyu Shu, Ph.D., Center for Surgery Research FF5, The Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, Ohio 44195.