Vaccination of Glioma Patients with Fusions of Dendritic and Glioma Cells and Recombinant Human Interleukin 12

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Abstract: Despite aggressive treatment, the median survival of patients with high-grade malignant astrocytoma is about 1 year. The authors investigated the safety and clinical response to immunotherapy using fusions of dendritic and glioma cells combined with recombinant human interleukin 12 (rhIL-12) for the treatment of malignant glioma. Fifteen patients with malignant glioma participated in this study. Dendritic cells were generated from peripheral blood. Cultured autologous glioma cells were established from surgical specimens in each case. Fusion cells were prepared from dendritic and glioma cells using polyethylene glycol. All patients received fusion cells intradermally on day 1. rhIL-12 was injected subcutaneously at the same site on days 3 and 7. Response to the treatment was evaluated by clinical observations and radiologic findings. No serious adverse effects were observed. In four patients, magnetic resonance imaging showed a greater than 50% reduction in tumor size. One patient had a mixed response. These results show that administration of fusion cells and rhIL-12 safely induces clinical antitumor effects in some patients with malignant glioma.

Key Words: immunotherapy, dendritic cells, glioma, brain tumor, interleukin 12

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M alignant astrocytoma is the most common primary brain tumor in adults. The median survival of patients with high-grade malignant astrocytoma is about 1 year, despite aggressive treatment with surgical resection, radiotherapy, and cytotoxic chemotherapy.[1] Novel therapeutic approaches are therefore needed to prolong survival. Immunotherapy is one such novel approach that has been investigated for different types of tumors, including brain tumors.

Dendritic cells (DCs) are professional antigen-presenting cells (APCs) that have a unique potency for activating T cells. DCs express high levels of major histocompatibility complex (MHC) and adhesion and costimulatory molecules.[2] Efficient isolation and preparation of both human and murine DCs is now possible.[3,4] Several methods that use DCs to induce antitumor immunity have been investigated, including DCs pulsed with proteins or peptides extracted from tumor cells,[5–7] DCs transfected with genes encoding tumor-associated antigens (TAAs),[8] DCs cultured with tumor cells,[9] and DCs fused with tumor cells.[10–12] Several of these approaches require a known TAA. However, since fusion cells (FCs) can induce antitumor immunity against unknown TAAs, and the TAAs of gliomas have not yet been identified, the use of FCs may offer a useful therapeutic approach for malignant gliomas. In this regard, vaccination with FCs has been shown to prolong the survival of mice with brain tumors.[11]

As reported previously, the results of a phase 1 clinical trial of FCs prepared with DCs and cultured autologous glioma cells indicated that this treatment safely induces immune responses.[13] However, we could not show a statistically significant treatment-associated response rate. Our study of a mouse brain tumor model showed that systemic administration of recombinant interleukin 12 (rIL-12) enhances the antitumor effects of FCs.[11] IL-12, originally known as natural killer cell stimulatory factor or cytotoxic lymphocyte maturation factor, enhances the lytic activity of natural killer (NK)/lymphokine-activated killer (LAK) cells, facilitates specific cytotoxic T-lymphocyte (CTL) responses, acts as a growth factor for activated T and NK cells, induces production of IFN-γ from T and NK cells, and acts as an angiogenesis inhibitor.[14] Based on data from the phase 1 trial and from our animal model, we began clinical trials of vaccine therapy using FCs and recombinant human IL-12 (rhIL-12). The present study describes the results of 15 patients with recurrent malignant glioma who were vaccinated with rhIL-12 and DCs fused with autologous glioma cells. The safety, feasibility, and immunologic and clinical responses of this approach are discussed.

METHODS

Patient Selection

Patients were selected using the following inclusion criteria: histologically proven glioblastoma, anaplastic astrocytoma, or other malignant gliomas according to the World Health Organization criteria; Karnofsky performance status of at least 70%; age at least 19; progression of their tumor despite

radiotherapy and/or chemotherapy; no antineoplastic chemotherapy or radiotherapy during the previous 4 weeks; residual tumors detectable by magnetic resonance imaging (MRI) or computed tomography (CT); and available cultured autologous tumor cells. All of the patients gave written informed consent, and the study was approved by the Ethical Committee of Jikei University. Treatment was carried out in the Department of Neurosurgery, Jikei University. Patient recruitment started in July 2001. Fifteen patients, ranging in age from 29 to 64 years (mean 45 years), were enrolled; their characteristics are summarized in Table 1. Steroids were not administered during the immunotherapy. The median Karnofsky performance scale was 90% (range 70–100%).

Generation of DCs From Peripheral Blood

DCs were separated from peripheral blood as described previously.[13] Briefly, peripheral blood mononuclear cells (PBMCs) were separated from peripheral blood (50 mL) using Ficoll-Hypaque density centrifugation. PBMCs were resuspended in RPMI1640 medium (Sigma, St. Louis, MO) and allowed to adhere to 24-well cluster plates. The nonadherent cells were removed after 2 hours at 37°C, and the adherent cells were subsequently cultured for 9 days in X-VIVO15 medium (BioWhittaker, Walkersville, MD) supplemented with 1% heat-inactivated autologous serum, 10 ng/mL recombinant human GM-CSF (Becton Dickinson, San Jose, CA), 10 U/mL recombinant human IL-4 (Becton Dickinson), and 10 ng/mL TNF-α (Becton Dickinson). The cultures were fed every third day and were split when necessary. Thereafter, the semiadherent and nonadherent cells were harvested by vigorous pipetting and used as DCs for fusion.

Generation of Cultured Glioma Cells From Surgical Specimens

Single cell suspensions of tumor cells were obtained by enzymatic digestion as described previously.[13] Briefly, each resected tumor was collected from surgery and handled under sterile conditions. Necrotic tissue, fatty tissue, clotted blood, and apparently normal tissue were removed and the remaining specimen was minced into small pieces using surgical blades. The chopped tissue was dissociated by mechanical stirring for 30 minutes at room temperature in a flask containing Dispase (10^3 U/mL; Goudou Inc, Tokyo, Japan). The resulting mixture was resuspended at 1×10^5 cells/mL in Dulbecco modified Eagle medium (Cosmo Bio) containing 10% fetal calf serum (FCS; Gibco, Gaithersburg, MD). The cells were cultured at 37°C in 5% CO_2.

Preparation of FCs

DCs were fused with glioma cells as described previously.[13] Briefly, DCs were mixed with lethally irradiated (300 Gy, Hitachi MBR-1520R, dose rate: 1.1 Gy/min) autologous glioma cells. The ratio of DCs and glioma cells ranged from 3:1 to 10:1 depending on the numbers of acquired DCs and glioma cells. Fusion was started by adding 500 μL of a 50% solution of polyethylene glycol (PEG; Sigma) dropwise for 60 seconds. The fusion was stopped by stepwise addition of serum-free RPMI medium. After washing three times with phosphate-buffered saline (PBS; Cosmo Bio), FCs were plated onto 100-mm Petri dishes in the presence of GM-CSF, IL-4, and TNF-α in RPMI medium for 24 hours.

Fusion efficiency was tested on an aliquot of the FCs. DCs and glioma cells were stained with PKH-2 (Sigma) and PKH-26 (Sigma), respectively, and then fused as described above. Fusion cells were resuspended in a buffer (1% BSA, 0.1% sodium azide in PBS) and analyzed using a FACScan flow cytometer (Becton Dickinson, San Jose, CA). Double positive cells were determined to be fusion cells. Fusion efficiency was calculated as follows: Fusion efficiency = double positive cells/total cells $\times$ 100 (%).

Study Design and Vaccination Schedule

The primary endpoints for the present study were to assess the feasibility and toxicity of vaccination with FCs and rhIL-12. The secondary endpoints were to assess immune, radiologic, and clinical responses induced by the vaccination procedure. The study protocol was approved by the ethical committee of Jikei University. All patients provided informed consent before treatment. All patients received the FCs on day 1. FCs, ranging from a total of 3.6 to 32.3 $\times$ 10^6 cells, were injected. FCs were suspended in 0.3 mL normal saline and then injected intradermally close to a cervical lymph node. rhIL-12 (30 ng/kg, provided by Wyeth Research, Cambridge, MA) was injected subcutaneously at the same site on days 3 and 7. This treatment was repeated every 2 weeks for 6 weeks. In the absence of progressive disease or grade 3 or 4 major organ toxicity, patients could receive a second 6-week course beginning 2 to 5 weeks after the last dose of rhIL-12 in course 1 (Fig. 1). Patients were monitored for immediate and delayed toxicities and the injection sites were examined at 48 hours. All toxicity was graded using the National Cancer Institute Common Toxicity Criteria. The response to the treatment was evaluated by clinical observations and radiologic findings. MRI or CT was performed to evaluate intracranial lesions before treatment and 6 and 10 weeks after the first immunization.

TABLE 1. Patient Characteristics

Case	Age/Sex (years)	Pathologic Diagnosis	Previous Therapy	Karnofsky Score (%)
1	40/M	AA	S, C, R	100
2	64/F	AOA	S, C, R	70
3	29/M	AA	S, R	100
4	60/M	GBM	S, C, R	90
5	40/F	AOA	S,	100
6	55/F	AA	S, C, R	80
7	32/M	GBM	S, C, R	70
8	50/M	AA	S, C, R	70
9	45/M	AA	S, C, R	100
10	46/M	GBM	S, C, R	100
11	42/F	GBM	S, C, R	100
12	55/M	GBM	S, C, R	90
13	56/F	GBM	S, C, R	90
14	32/M	AA	S, R	100
15	49/M	AA	S, C, R	100

GBM, glioblastoma multiforme; AA, anaplastic astrocytoma; AOA, anaplastic oligoastrocytoma; S, surgery; C, chemotherapy; R, radiotherapy; ND, not done.

FIGURE 1. Vaccination schedule. FCs were injected intradermally close to a cervical lymph node on day 1. rhIL-12 was injected subcutaneously at the same site on days 3 and 7. This cycle was repeated every 2 weeks for 6 weeks (upper panel). In the absence of progressive disease or grade 3 or 4 major organ toxicity, patients could receive a second 6-week course beginning 2 to 5 weeks after the last dose of rhIL-12 during course 1 (lower panel).
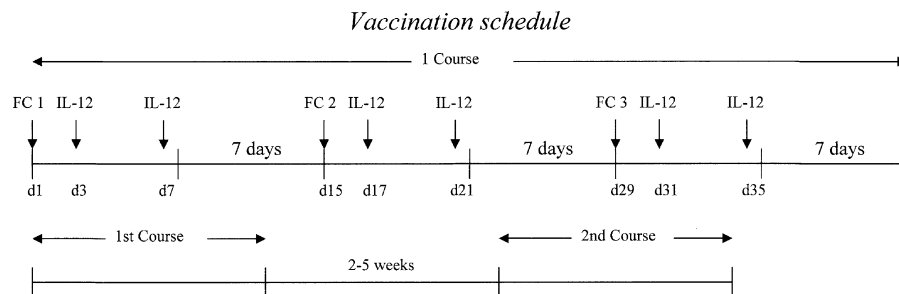
Patients subsequently underwent MRI or CT every 2 months. Tumor size was estimated as the volume of the region of abnormal enhancement observed on MRI or CT using computer software Zed View (LEXI Co. Ltd, Tokyo, Japan). Response was classified as one of the following four categories: complete response, defined as disappearance of the entire tumor; partial response, defined as a reduction of 50% or more in tumor size; no change, defined as either a decrease of less than 50% or an increase of less than 25% in tumor size; and progressive disease, defined as an increase of 25% or more in tumor size.

Cell Surface Analysis

PBMCs were resuspended in 1% bovine serum albumin (BSA), 0.1% sodium azide in PBS, and stained with anti-human CD3, CD4, CD8, CD16, CD19, and CD56 monoclonal antibodies (Pharmingen, San Diego, CA) for 30 minutes at 4°C. Stained cells were washed with PBS and analyzed using a FACScan flow cytometer.

^{51}Cr Release Assay

The cytolytic activity of peripheral blood lymphocytes (PBLs) was tested in vitro in a standard ^{51}Cr release assay. Single cell suspensions of PBLs were washed and resuspended in 10% FCS-RPMI at a density of 1×10^7/mL in 6-well plates (day 0). Recombinant human IL-2 (10 U/mL) (provided by Shionogi, Osaka, Japan) was added to the cultures every other day. Four days after culture initiation, cells were harvested and CTL activity was determined. Target cells were labeled by incubation with ^{51}Cr for 90 minutes at 37°C, then co-cultured with effector lymphocytes for 4 hours. The effector:target ratio was 80:1 due to the limited number of lymphocytes. All determinations were made in triplicate and percentage lysis was calculated using the formula: (experimental cpm − spontaneous cpm/maximum cpm − spontaneous cpm) × 100%.

Intracellular Staining for IFN-γ

Single cell suspensions of PBLs were washed and resuspended in 10% FCS-RPMI at a density of 1×10^7/mL with irradiated autologous tumor cells (1×10^6/mL) in six-well plates (day 0). Recombinant human IL-2 (10 U/mL) was added to the cultures every other day. Four days after culture initiation, cells were harvested and CTL activity was determined. PBLs were stained with both labeled anti-human CD8 and anti-human IFN-γ antibodies (Pharmingen) using fix and perm cell permeabilization reagents (CALTAG Laboratory, Burlingame, CA) according to the manufacturer's instructions.

Stained cells were washed with PBS and analyzed using a FACScan flow cytometer.

Light Microscopic and Immunohistochemical Analysis

In two patients (patients 1 and 6), tumors were resected after vaccination for the purpose of internal decompression. In addition to routine light microscopic assessment of formalin-fixed, paraffin-embedded sections stained using hematoxylin and eosin, immunopathologic examinations were also performed. Serial sections of the paraffin blocks were immunostained using an avidin-biotin immunoperoxidase technique. Tumor infiltrating lymphocytes were detected using anti-CD4 and anti-CD8 antibodies (Becton Dickinson).

RESULTS

Vaccine Preparation and Characterization

To assess fusion efficiency (FE), DCs and glioma cells were stained with PKH 2 and PKH 26, respectively, and fused with PEG. Double positive cells were determined to be FCs. A representative case is shown in Figure 2. The percentage of double positive cells (FCs) was 66.2%, while the PKH2 positive cells (unfused tumor cells) were less than 1%, suggesting that cells injected into patients consisted predominantly of FCs and unfused DCs. Double positive cells were not detected after the fusion without PEG (data not shown).

Vaccine Administration

Five patients received at least two courses of intradermal vaccination with FCs and rhIL-12. Three courses of vaccination were given to patients 1 and 9. The total number of inoculated FCs was 13.7×10^6 cells (mean), ranging from 3.6×10^6 to 3.2×10^7 (Table 2). The total dose of rhIL-12 was 15.7 μg (mean), ranging from 6.0 to 37.8 μg (see Table 2).

Toxicity of Vaccination

Vaccination with FCs and rhIL-12 was well tolerated in all patients. We observed no serious adverse effects, clinical signs of autoimmune reaction, or substantial changes in the results of routine blood tests, including absolute lymphocyte count. Transient grade 1 fever occurred in four patients (patients 1, 2, 9 and 11). In patient 7, general convulsion occurred once during the second course of the treatment; it remains unclear whether there was any causal relationship between the convulsion and immunotherapy. In 13 cases, erythema and induration were observed at the injection site after the second and/or the third immunization with FCs during the first course,
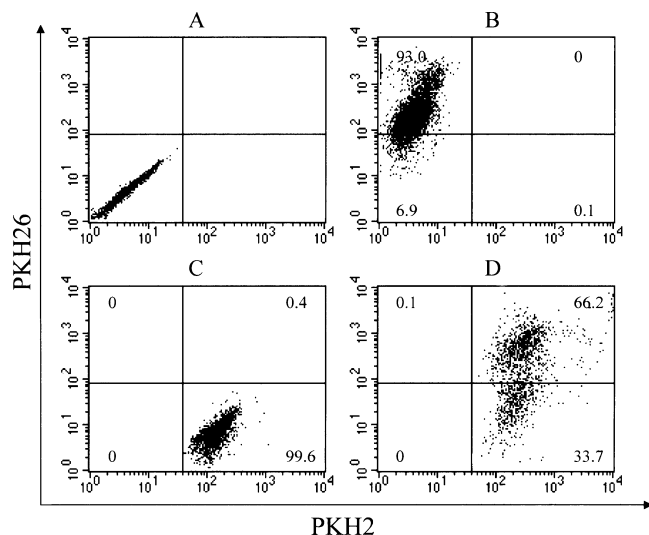
FIGURE 2. Analysis of fusion efficiency using FACScan. A, Negative control. B, PKH 26 was incorporated into glioma cells; 93.0% of glioma cells were positive for PKH 26. C, PKH 2 was incorporated into DCs; 99.6% of DCs were positive for PKH 26. D, Stained glioma cells and DCs were fused with PEG. Double positive cells (66.2%) were determined to be fusion cells. The numbers show the percentage of cells. Vertical axis: PKH 26, horizontal axis: PKH 2.

suggesting a delayed-type hypersensitivity reaction. During the second course, all patients developed injection site erythema and induration. Although transient liver dysfunction and leukocytopenia occurred in six and seven patients, respectively, in none of the patients was the treatment abandoned due to adverse effects.

Clinical Responses

Clinical response data are listed in Table 2. Four patients experienced deterioration in symptoms. In patients 4, 10, and 12, level of consciousness worsened at the end of first course of vaccination. In patient 6, hemiparesis worsened during the study. In these four patients, therapy was discontinued because of the need to administer steroids. In the remaining 11 patients, clinical symptoms were not observed before treatment and did not worsen during therapy. Radiologic findings showed that four patients had partial responses (patients 1, 2, 9, and 15; Figs. 3 and 4). One patient had a mixed response (patient 3) and two patients exhibited stable disease (patients 5 and 7).

A representative case is shown (see Fig. 3). In patient 1, the tumor recurred 2 months after the initial operation, despite postoperative chemotherapy and radiotherapy. FCs without rhIL-12 were administered, but there was no effect on tumor growth. Therefore, combination therapy with FCs and rhIL-12 was initiated in July 2001. The size of tumor on the T1-weighted image decreased 70.2% by 4 months after the first immunization. The high-intensity area around the tumor on the T2-weighted image decreased 4 weeks after the first immunization. Recurrence of the tumor required surgical removal 6 months after initial immunization (January 2002). Following culture of this specimen, one course of the vaccination with FCs prepared with DCs and newly established glioma cells was administered with rhIL-12. However, therapy was discontinued because of deterioration in symptoms and progression of tumor size.

In patient 3, the high-intensity area around the tumors on T2-weighted imaging decreased 6 weeks after the first immunization (Fig. 5), although a reduction on T1-weighted imaging was not apparent (data not shown). This patient was therefore categorized as a mixed response.

TABLE 2. Results of FCs and rhIL-12 Immunotherapy

Case	No. of Courses	Total Amount of FCs ($\times 10^6$)	Total Amount of IL-12 (µg)	Clinical Response After 8 weeks	Radiologic Findings	Duration of Response (months)	Outcome (months†)
1	3	32.3	37.8	SD	PR	6	DD (12)
2	1	12.0	8.1	SD	PR	7	PD (14)
3	2	16.4	12.6	SD	MR	12*	SD (18)
4	1	12.4	13.5	PD	PD	—	DD (3)
5	2	18.6	18.0	SD	NC	12*	PD (18)
6	1	3.6	6.0	PD	PD	—	DD (8)
7	2	8.1	21.0	SD	NC	4	DD (12)
8	1	7.4	11.7	SD	PD	—	PD (12)
9	3	40.4	12.6	SD	PR	6	PD (12)
10	1	11.3	12.6	PD	PD	—	DD (3)
11	1	10.3	6.6	SD	PD	—	PD (9)
12	1	5.2	4.2	PD	PD	—	PD (9)
13	1	6.5	9.0	SD	PD	—	PD (8)
14	1	22.9	12.6	SD	PD	—	PD (6)
15	1	11.0	12.6	SD	PR	3	SD (3)

SD, stable disease; PR, partial response; PD, progressive disease; DD, died of disease; NC, no change; MR, mixed reaction; ND, not done; F, fever; E, erythema; I, induration.
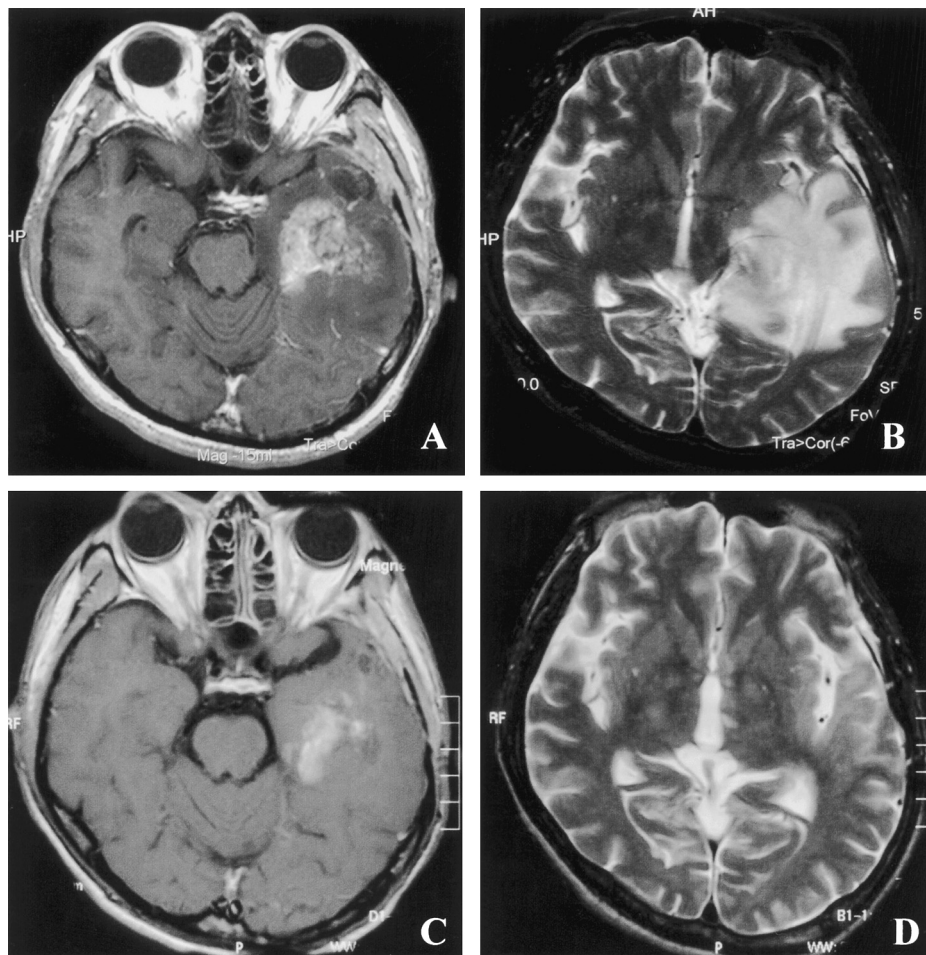*Response continues to date.
†Period after the initial vaccination.

FIGURE 3. MRI of patient 1 shows that the tumor recurred 2 months after the first operation. Inoculation of FCs did not inhibit tumor growth. After combination therapy using FCs and rhIL-12, the high-intensity area around the tumor on the T2-weighted image and the size of tumor on the T1-weighted image decreased remarkably. T1-weighted (A) and T2-weighted (B) images of recurrent tumor. T1-weighted (C) and T2-weighted (D) images after immunization with FCs and rhIL-12.

Pathologic Responses

In patients 1 and 6, operations to remove growing tumors were performed 2 months and 2 weeks after the last immunization, respectively. In both cases, many larger tumor cells containing multiple nuclei and extended cytoplasm were observed in the recurrent tumor specimens compared with that in the primary tumors (Fig. 6). These patients also exhibited a robust infiltration of CD8$^+$ T lymphocytes in areas of the tumor, which was not apparent on tumor specimens obtained before vaccination (data not shown). By contrast, infiltration of CD4$^+$ T-cells was not apparent.

Immunologic Responses

The surface phenotype of PBLs was investigated using FACScan before and after immunotherapy in seven patients. We analyzed the expression of CD3, 4, 8, 16, 19, and 56. The percentage of each surface phenotype before and after therapy (data not shown) did not change significantly. We then investigated whether the immunotherapy affected the response of PBLs against autologous glioma cells. The cytolytic activity of PBLs was tested in vitro using a standard ^{51}Cr release assay in patients 1 to 8. PBLs were separated from blood taken before and 8 to 10 weeks after first immunization. In two patients (patients 1 and 2), cytolytic activity against autologous tumor cells increased after treatment, while in other patients cytolytic activity was almost nonexistent after treatment (Fig. 7). In patient 6, the cytolytic activity after the treatment was lower than that before the treatment. In patients 9 to 15, the cytolytic activity of PBLs was tested in vitro using intracellular staining for IFN-γ. In patient 15, the percentage of double positive cells increased after the treatment, while in other patients the percentage of double positive cells was almost zero both before and after the treatment (Fig. 8).

DISCUSSION

Genetically engineered glioma cells can be used as APCs for vaccination against gliomas, but the antitumor effect is insufficient to eradicate established brain tumors in the mouse model.[15,16] However, an intradermal injection of FCs prepared with DCs and glioma cells prolongs the survival of mice with brain tumors.[11] Based on these experimental data, we started a clinical trial of immunotherapy for gliomas using FCs. As reported previously, the results of a phase 1 clinical trial of FCs from DCs and cultured autologous glioma cells indicated that this treatment safely induces antitumor immune responses.[14] However, we could not show a statistically significant treatment-associated response rate. Our study in a mouse brain tumor model showed that systemic administration
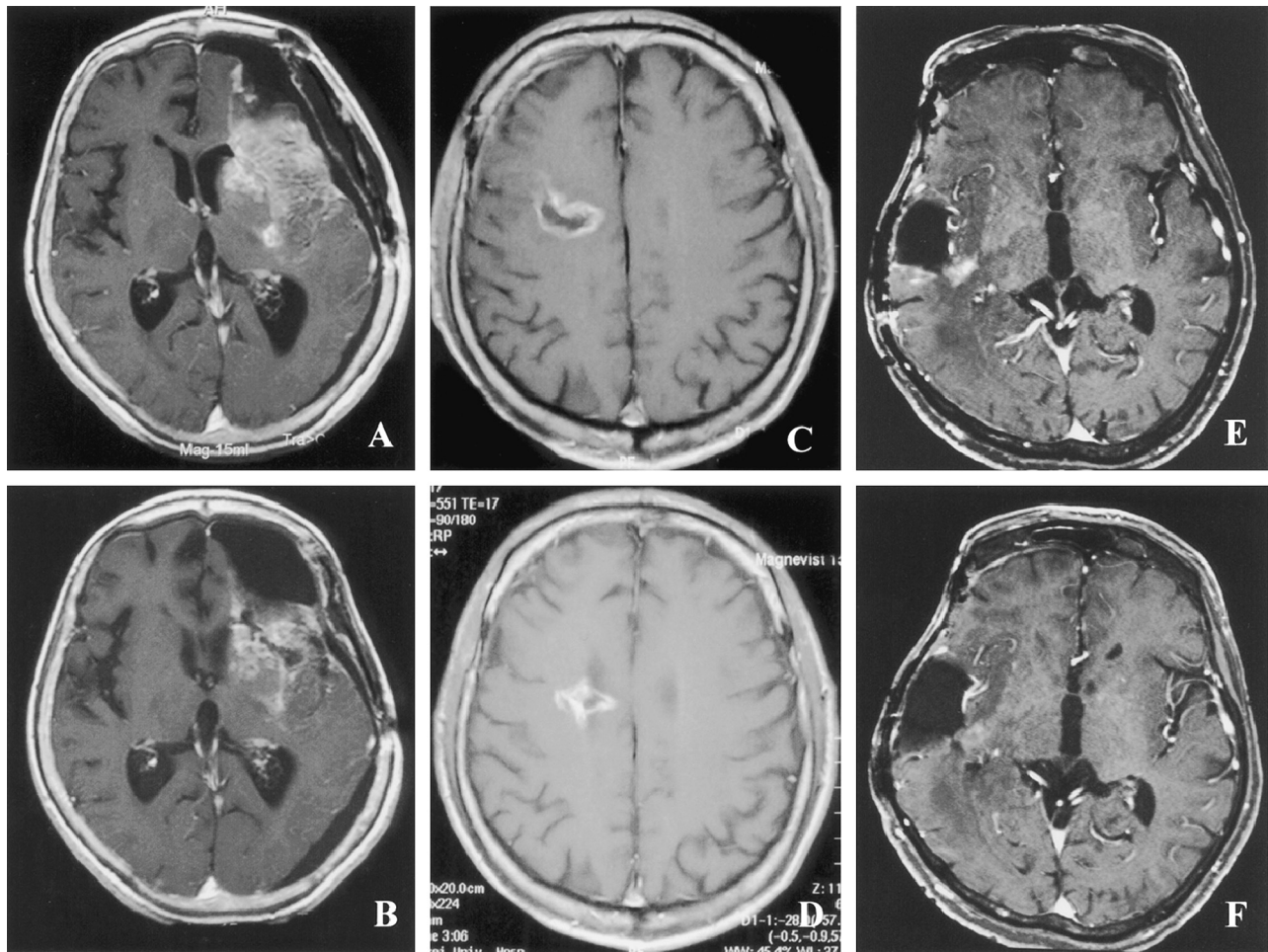
FIGURE 4. MRI findings showed that the size of tumors on the T1-weighted image decreased. A, C, and E, T1-weighted images of recurrent tumor in patients 2, 9 and 15, respectively. B, D, and F, T1-weighted images after immunization with FCs and rhIL-12 in patients 2, 9 and 15, respectively.
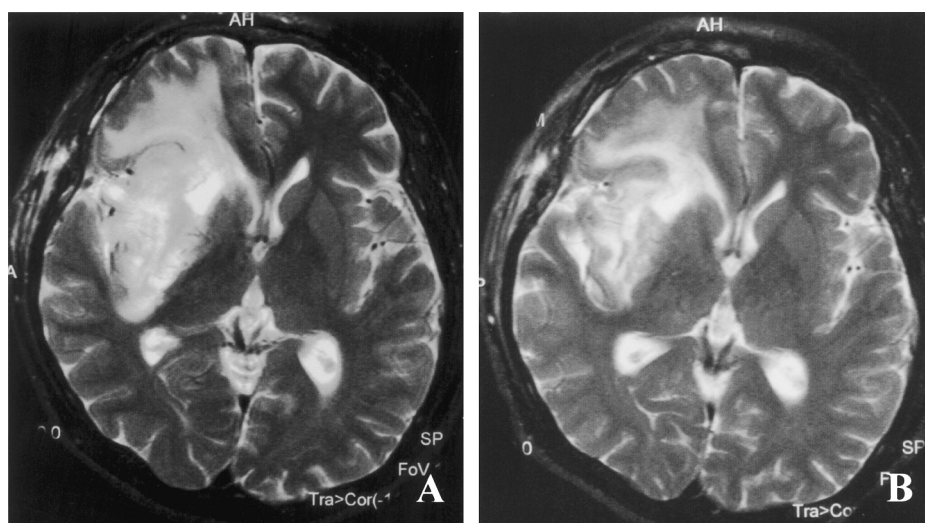

FIGURE 5. MRI of patient 3 shows the reduction in the high-intensity area around the tumors on the T2-weighted image. A, T2-weighted image before immunization. B, T2-weighted image after immunization with FCs and rhIL-12.
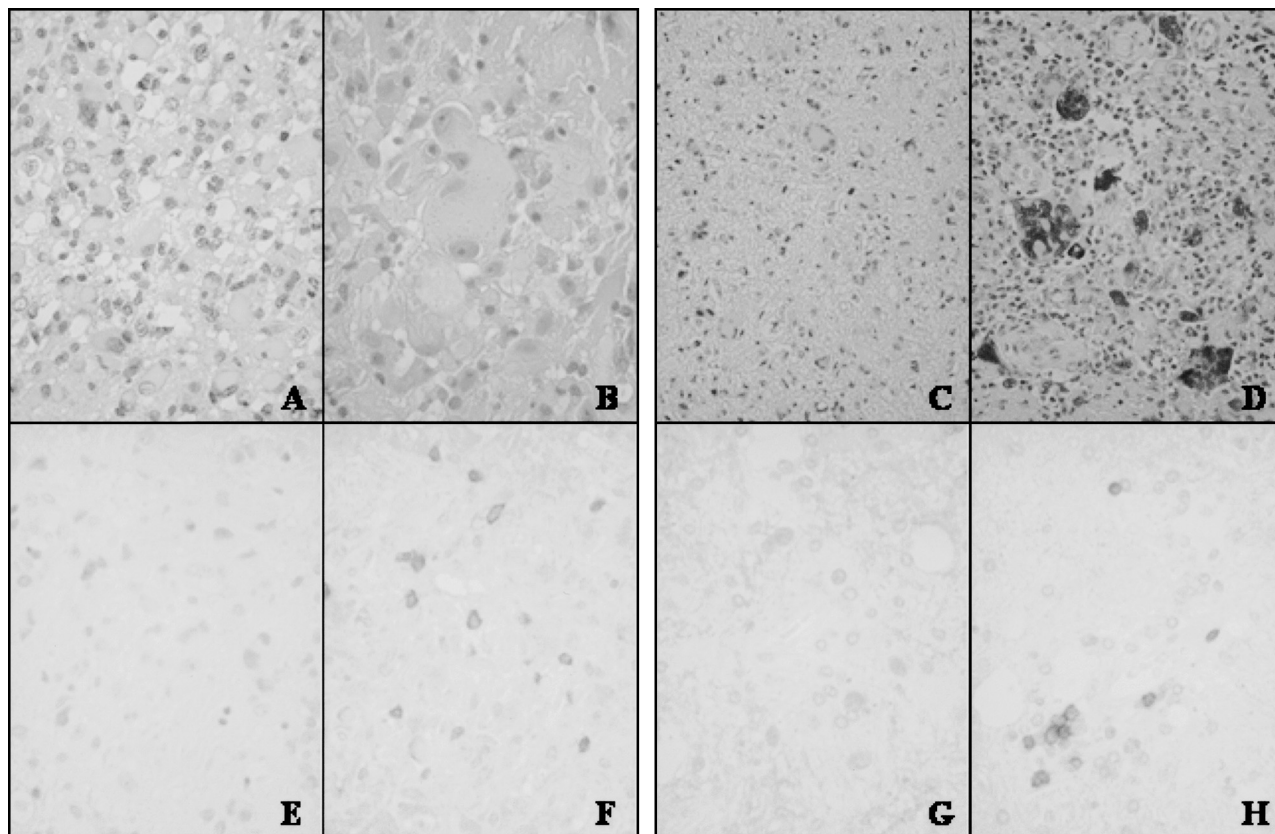
FIGURE 6. Pathologic findings in tumor specimens. Many larger tumor cells containing multiple nuclei and wide cytoplasm were observed in recurrent tumor specimens compared with primary tumors. A robust CD8^{+}, but not CD4^{+}, T-lymphocyte infiltration was observed in areas of the tumor. Hematoxylin-and-eosin staining of primary and recurrent tumors in patient 1 (A, B) and patient 6 (C, D). Immunohistochemical staining of recurrent tumor specimens with anti-CD4 and anti-CD8 monoclonal antibodies in patient 1 (E, F) and patient 6 (G, H).

of rIL-12 enhances the antitumor effects of FCs. Based on the data from our phase 1 trial and the animal study, we initiated a clinical trial of vaccine therapy using FCs and rhIL-12.

Treatment efficacy for this method was better than that of FCs alone. These data are compatible with the results from the experiments in a mouse brain tumor model in which administration of FCs and rIL-12 markedly prolonged the survival of mice with brain tumors compared with FCs or rIL-12 alone.[11] In the mouse brain tumor model, many CD4^{+} and CD8^{+} T cells were detected in the tumors of vaccinated mice. In the present study, pathologic findings of a recurrent tumor resected after the immunization showed infiltration by CD8^{+}, and not CD4^{+}, T lymphocytes. In ^{51}Cr release assays, antitumor CTL activity was increased after vaccination in two patients with a partial response (patients 1 and 2). Conversely, in patients 4 and 6, CTL activity against autologous glioma cells decreased after the treatment. In both cases, therapy was discontinued because of deterioration in symptoms and progression of tumor size. Potential reasons for the decrease in immunologic response are tumor progression that suppresses immunologic reactivity, and tolerance against the tumor induced by the vaccination.

Interestingly, in two patients (patients 1 and 3), the high-intensity area around the tumor on the T2-weighted image decreased 4 weeks after the first immunization; in patient 1, this finding was followed by a reduction in the tumor on the T1-weighted image. We reported previously, in one of eight patients treated with FCs alone, that the high-intensity area decreased around the tumor on the T2-weighted image.[14] High-intensity areas on T2-weighted images are caused by glioma cells migrating into the tumor periphery. Thus, induction of antitumor immunity may result in death or inhibition of the activity of migrating tumor cells in the periphery. Likewise, vascular permeability may be affected and thereby contribute to a reduction in the tumor volume.

rhIL-12 has been investigated in several clinical trials in patients with malignant tumors.[17] Common toxicities included fever, chills, pulmonary toxicity, depression, and gastrointestinal bleeding; laboratory changes included anemia, leukopenia, and liver dysfunction. The maximum tolerated rhIL-12 dose was previously reported as 500 to 1,000 ng/kg; in the present study, the rhIL-12 dose was 30 ng/kg. Low-dose rhIL-12 was administered because the FCs and rhIL-12 in combination may have synergistically induced adverse effects. However, we observed no serious adverse effects, such as autoimmune responses.

The advantages of the treatment outlined in the present study include the following: FCs can be used to induce
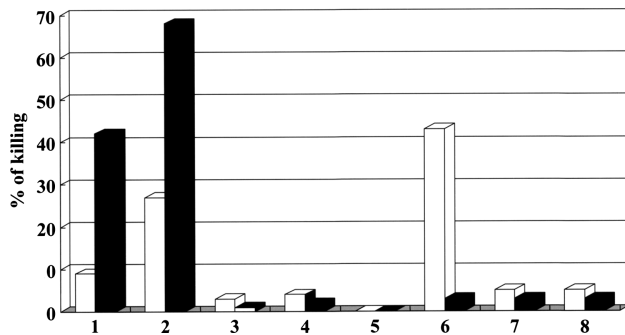
FIGURE 7. Cytolytic activity of PBLs against autologous glioma cells. PBLs were separated from blood taken before (black bar) and 8 to 10 weeks after first immunization (white bar). In two patients (patients 1 and 2), cytolytic activity against autologous tumor cells increased after treatment, while in other patients cytolytic activity was almost nonexistent after treatment. In patient 6, the cytolytic activity after the treatment was lower than that before the treatment. The effector:target ratio was 80:1.

antitumor immunity against unknown TAAs, and there is no evidence for induction of autoimmune responses. One of the disadvantages is that cultured glioma cells are needed. Since we fused DCs with cultured glioma cells, our method avoids fusion with normal cells. However, in the present study, glioma cells established from specimens taken during the initial operation were used as a fusion partner. TAAs of recurrent tumors may not be the same as those of cultured tumor cells, resulting in an "escape phenomenon" in which CTLs induced by FCs kill only tumor cells expressing the same TAAs as

those of the cultured tumor cells. Therefore, the escape phenomenon may have been responsible for disease progression in patients on our trial.

These results show that administration of FCs and rhIL-12 safely induces clinically antitumor effects in some patients with malignant glioma. A combination of FCs and high-dose rhIL-12 (60–100 ng/kg) may result in better outcomes. Therefore, as no serious adverse effects have been observed to date, a dose escalation study is planned.

ACKNOWLEDGMENTS

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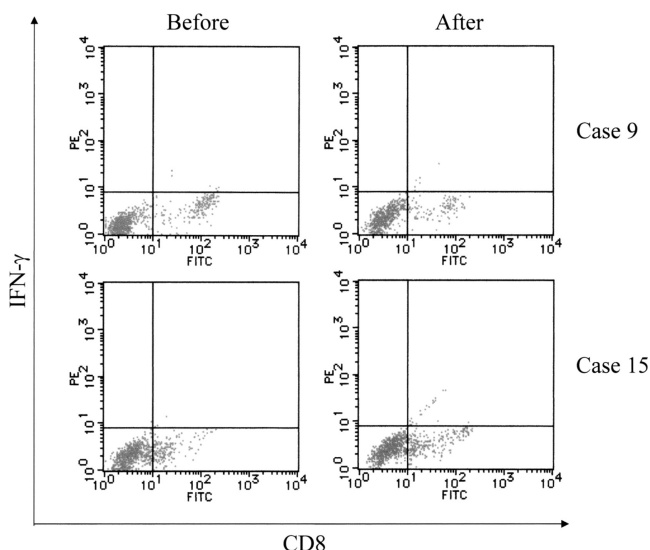

FIGURE 8. Cytokine flow cytometry for detection of IFN-γ-expressing CD8+ T lymphocytes in the peripheral circulation of patients before and after the treatment. Representative cases are shown (patients 9 and 15). In patient 15, the percentage of double positive cells increased after the treatment.