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ORIGINAL ARTICLE

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Results of a phase I clinical trial of vaccination of glioma patients with fusions of dendritic and glioma cells

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Abstract Several reports of clinical trials of immunotherapy using dendritic cells have been published to date. In this study, we investigated the safety and clinical response of immunotherapy with fusions of dendritic and glioma cells for the treatment of patients with malignant glioma. Eight patients with malignant glioma, ranging in age from 4 to 63 years old, 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 of dendritic and glioma cells were prepared with polyethylene glycol, and the fusion efficiency ranged from 9.2 to 35.3% (mean, 21.9%). All patients received the fusion cells every three weeks for a minimum of 3, and a maximum of 7, immunizations. Fusion cells were injected intradermally, close to a cervical lymph node. The percentage of CD16- and CD56-positive cells in peripheral blood lymphocytes slightly increased after immunization in 4 out of 5 cases investigated. Peripheral blood mononuclear cells were incubated with irradiated autologous glioma or U87MG cells and supernatants were harvested. In 6 cases analyzed, the concentration of interferon-γ in the supernatant increased after immunization. Clinical results showed that there were no serious adverse effects and two partial responses. Although the results of the phase I clinical trial of fusion cells indicated that this treatment safely induced immune responses, we were unable to establish a statistically significant treatment-associated response rate, due to the limited sample population. Therefore, further evaluation of the role of adjuvant cytokines is necessary.

Keywords Immunotherapy · Astrocytomas · Malignant gliomas · Dendritic cells · Fusion

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Introduction

Malignant astrocytoma is the most common primary brain tumor in adults. The median survival time of patients with high-grade malignant astrocytomas is about one year, despite aggressive treatment that includes surgical resection, radiotherapy and cytotoxic chemotherapy. Therefore, novel therapeutic approaches are needed to prolong survival. Immunotherapy is one such novel approach that has been investigated using different types of tumors, including brain tumors.

Based on the hypothesis that genetically engineered tumor cells will be effective antigen-presenting cells (APCs) of tumor-associated antigens (TAAs), many of the recently developed immunotherapeutic approaches against cancer, including malignant astrocytomas, have used genetically engineered tumor cells to express cytokines or costimulatory molecules that enhance immune responses [2, 5, 9, 13, 30]. However, tumor cells are poor APCs, because they lack costimulatory molecule expression and secretion of immunosuppressive cytokines, such as transforming growth factor-β (TGF-β) and vascular endothelial growth factor (VEGF) [6, 8]. Dendritic cells (DCs) are professional APCs that have a unique potency for activating T cells. DCs express high levels of major histocompatibility complex (MHC), adhesion and costimulatory molecules [27]. The efficient isolation and preparation of both human and murine DCs are now possible [24, 26]. Several methods that use DCs for the induction of antitumor immunity have been investigated to date: DCs pulsed with proteins or peptides extracted from tumor cells [23, 28, 32], DCs transfected with genes encoding TAAs [29], DCs cultured with tumor cells [4], and DCs fused with tumor

cells [10, 18, 19, 31]. Clinically, DCs pulsed with tumor lysate or tumor peptides were generally used [14, 22, 24]. However, since (1) fusion cells (FCs) can induce antitumor immunity against unknown TAAs and (2) the common TAAs of gliomas have not yet been identified, the use of FCs may offer a potentially therapeutic approach for malignant gliomas, particularly since vaccination with FCs prolonged the survival of mice with brain tumors [1].

Based on these experimental findings, clinical trials of vaccine therapy with FCs have begun. In the present study, we describe the vaccination of eight malignant glioma cases with dendritic cells fused with autologous glioma cells. The safety, feasibility and immunological response of this approach are discussed.

Materials and methods

Patient selection for treatment by vaccination with fusion cells

Patients were selected using the following inclusion criteria. Patients had: (1) histologically proven glioblastoma, anaplastic astrocytoma, or other malignant gliomas according to the World Health Organization (WHO) criteria; (2) progression of their tumor, despite radiotherapy and/or chemotherapy; (3) no antineoplastic chemotherapy or radiotherapy during the previous 4 weeks; (4) residual tumors detectable using magnetic resonance imaging (MRI) or computed tomography (CT); (5) available primary-cultured autologous tumor cells. Treatment was carried out at the Department of Neurosurgery, Jikei University. Patients' recruitment started in August 1999, and the study is ongoing at the present time (June 2000). Eight patients, ranging in age from 4 to 63 years old (mean, 38 years), were enrolled and their characteristics are summarized in Table 1. A steroid was administered in 5 cases during the immunotherapy. The median Karnofsky performance scale was 70%, ranging from 30 to 100%.

Generation of dendritic cells from peripheral blood

Peripheral blood mononuclear cells (PBMCs) were separated from peripheral blood (50 ml) using Ficoll-Hypaque density centrifugation. PBMCs were resuspended in RPMI-1640 (Sigma, St. Louis, Mo.) and allowed to adhere to 24-well cluster plates. The nonadherent cells were removed after 2 h at 37 °C, and the adherent cells were subsequently cultured for 7 days in RPMI-1640 medium supplemented with 1% heat-inactivated autologous serum, 50 mM 2-mercaptoethanol (Sigma), 2 mM glutamate (Cosmo Bio, Japan), 100 U/ml penicillin (Sigma), 100 mg/ml streptomycin (Sigma), 10 ng/ml recombinant human granulocyte-macrophage colony stimulating factor (GM-CSF; Becton Dickinson, San Jose, Calif.), 30 U/ml recombinant human interleukin-4 (IL-4; Becton Dickinson) and 20 ng/ml tumor necrosis factor-α (TNF-α; Becton Dickinson). The cultures were fed every third day and were split when necessary. Thereafter, the semi-adherent and nonadherent cells were harvested by vigorous pipetting and used as dendritic cells for fusion.

Generation of cultured glioma cells from surgical specimens

Single-cell suspensions of tumor cells were obtained by enzymatic digestion. Briefly, each resected tumor was collected from surgery and transported in a sterile fashion. 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 min at room temperature in a flask containing dispase (10^3 U/ml; Goudou, Tokyo, Japan). The resulting mixture was resuspended at a final concentration of 1×10^5 cells/ml in Dulbecco's MEM (Cosmo Bio) with 10% fetal calf serum (FCS; Gibco, Gaithersburg, Md.). The cells were cultured at 37 °C in 5% CO_2.

Preparation of fusion cells

DCs were fused with glioma cells, as described previously [10]. Briefly, DCs were mixed with lethally-irradiated (200 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 upon the numbers of acquired DCs and glioma cells. Then, fusion was started by adding 500 ml of a 50% solution of polyethylene glycol (PEG; Sigma) dropwise for 60 s. The fusion was stopped by stepwise addition of serum-free RPMI-1640 medium. After washing 3 times with phosphate-buffered saline (PBS; Cosmo Bio), FCs were plated in 100-mm petri dishes in the presence of GM-CSF, IL-4 and TNF-α in RPMI-1640 medium for 48 h.

Fusion efficiency was investigated as follows. DCs and glioma cells were stained with PKH-2 (Sigma) and PKH-26 (Sigma), respectively, according to the manufacturer's instructions. Immediately, those cells were fused as described above. Fusion cells were resuspended in PBS buffer containing 1% bovine serum albumin (BSA) and 0.1% sodium azide, and analyzed using a FACScan flow cytometer (Becton Dickinson, San Jose, Calif.). Double-positive cells were determined to be fusion cells. Fusion efficiency was calculated as follows: fusion efficiency (%) = (double-positive cells/total cells) $\times100$.

Design of the phase I trial of fusion cell therapy

The study protocol was approved by the ethical committee of Jikei University. All patients provided informed consent before treatment. All patients received the FCs made from autologous DCs and autologous glioma cells every three weeks for a minimum of 3, and maximum of 7, immunizations. As many FCs as possible were injected, ranging from 2.4×10^6 to 8.7×10^6 cells a time as DCs. FCs

Table 1 Patient characteristics (*GBM* glioblastoma multiforme, *AA* anaplastic astrocytoma, *AO* anaplastic oligodendroglioma, *S* surgery, *C* chemotherapy, *R* radiotherapy, *ND* not done)

Case	Age (years)/sex	Pathological diagnosis	Previous therapy	Karnofsky score (%)	MHC expression on autologous glioma cells	
					Class I/+IFN-γ	Class II/+IFN-γ
1	41/M	GBM	S, C, R	70	++/+++	/
2	56/M	AA	S, C, R	90	+++/+++	/++
3	33/F	GBM	S, C, R	90	++/+++	/
4	37/M	GBM	S, C, R	70	++/+++	/+
5	63/M	GBM	S, C, R	90	++/++	/+
6	36/M	AO	S, C, R	100	++/++	/
7	37/M	GBM	S, C, R	50	++/+++	/
8	4/M	AA	S, C, R	30	ND	ND

were drawn in 0.3 ml normal saline and were injected intradermally, close to a cervical lymph node. Patients were monitored for immediate and delayed toxicities and the injection sites were examined for any clinical evidence at 48 h. All toxicity was graded using the National Cancer Institute Common Toxicity Criteria. The response to the treatment was evaluated by clinical observations and radiological findings. MRI or CT was performed to evaluate intracranial lesions before, and 8 weeks after, the first immunization. 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. Response was classified into one of the following four categories: (1) complete response (CR), defined as disappearance of the entire tumor for a period of at least 4 weeks; (2) partial response (PR), defined as a reduction of 50% or more in tumor size for at least 4 weeks; (3) no change (NC), defined as either a decrease of less than 50 % or an increase of less than 25% in tumor size for at least 4 weeks; (4) progressive disease (PD), defined as an increase of 25% or more in tumor size.

Cell surface analysis

PBMCs were separated from the peripheral blood of patients, as mentioned above. PBMCs were resuspended in PBS buffer containing 1% BSA and 0.1% sodium azide, and stained with anti-human CD3, CD4, CD8, CD16, CD19 and CD56 monoclonal antibodies (Pharmingen, San Diego, Calif.) for 30 min at 4 °C. Stained cells were washed with PBS and analyzed using a FACScan flow cytometer.

DCs and FCs were resuspended in PBS buffer containing 1% BSA and 0.1% sodium azide, and stained with anti-human HLA-ABC, anti-human HLA-DR, anti-CD80, anti-CD86 and anti-CD83 monoclonal antibodies (Pharmingen) for 30 min at 4 °C. Stained cells were washed and analyzed using a FACScan flow cytometer.

Tumor cells were resuspended in PBS buffer containing 1% BSA and 0.1% sodium azide, and stained with anti-human HLA-ABC and anti-human HLA-DR monoclonal antibodies (Pharmingen) for 30 min at 4 °C. Stained cells were washed and analyzed using a FACScan flow cytometer.

ELISA

PBMCs (1×10^6/well) were cultured with irradiated (200 Gy) autologous glioma or U87 MG cells (1×10^4/well; obtained from ATCC, Rockville, Md.) for 48 h in a 96-well plate. Supernatants were harvested and frozen at –80 °C until analysis. The production of interferon-γ (IFN-γ) in the supernatants was measured by a commercially available ELISA kit (R&D Systems, Minneapolis, Minn.). Each assay was performed according to the manufacturer's instructions. The lower detection limit was 4 pg/ml. All samples and standards were run in triplicate.

Results

Vaccine preparation and characterization

Surface phenotypes of dendritic cells established from peripheral blood and FCs were analyzed using FACScan. DCs in this study were positive for MHC class I, class II, CD80 and CD86, and weakly positive for CD83. FCs, too, were positive for MHC class I, class II, CD80, CD86 and CD83. The expression of these surface markers on FCs was lower than those on DCs. The representative case (case 1) is shown in Fig. 1.

An analysis of the characters of established glioma cells showed that all cultured glioma cell lines were positive for MHC class I, but negative for MHC class II (Table 1), CD80, and CD86 (data not shown), constitutively. We then stimulated cultured glioma cells with IFN-γ at a concentration of 1000 U/ml. Glioma cells were harvested after 24 h and analyzed using FACScan. IFN-γ induced the expression of MHC class II in 3 of 7 cell lines. MHC class I expression was enhanced in four cell lines. IFN-γ treatment showed no effect on the expression of CD80 or CD86 (data not shown).

Fusion efficiency (FE) was investigated. Immediately after staining dendritic and glioma cells with PKH 2 and PKH 26, respectively, both cells were fused with PEG. Double-positive cells were determined to be fusion cells. FE was analyzed in 6 of 8 cases. FE ranged from 9.2 to 35.3% (mean, 21.9 %; Table 2). The representative case (case 2) is shown in Fig. 2. No significant correlation was found between the FE and the ratio of tumor cells and DCs at fusion (data not shown). Double-positive cells were not detected after the fusion procedure was undertaken without PEG (data not shown).

Vaccine administration

Intradermal vaccination with FCs was performed at least three times, with the exception of cases 7 and 8. Mean administration was 4.5 times, ranging from 1 to 9. In cases 7 and 8, therapy was discontinued due to a deterioration in symptoms between the first and second immunization. The total number of inoculated FCs was 11.9×10^6 cells (mean), ranging from 4.6×10^6 to 2.5×10^7 (Table 2).

Immunological responses

The surface phenotype of peripheral blood lymphocytes (PBL) was investigated using FACScan before and after immunotherapy. We analyzed the expression of CD3, CD4, CD8, CD16, CD19 and CD56 in 5 of 8 cases. No constant change in the percentage of CD3, CD4, CD8 or CD19 was detected in the period before and after therapy, whereas the percentage of CD16- and CD56-positive cells slightly increased in 4 of 5 cases analyzed (Table 3).

We then investigated whether the immunotherapy affected the response of PBMCs against autologous glioma cells. PBMCs were separated from blood taken before, and 8–10 weeks after, the first immunization. PBMCs were incubated with irradiated autologous glioma or U87MG cells for 48 h and supernatants were harvested. The concentration of IFN-γ was measured using ELISA. Interestingly, in all cases analyzed, the concentration of IFN-γ was high in supernatants of PBMCs after the immunization, compared with those before the immunization (Table 4). The concentration of IFN-γ was elevated in supernatants of PBMCs

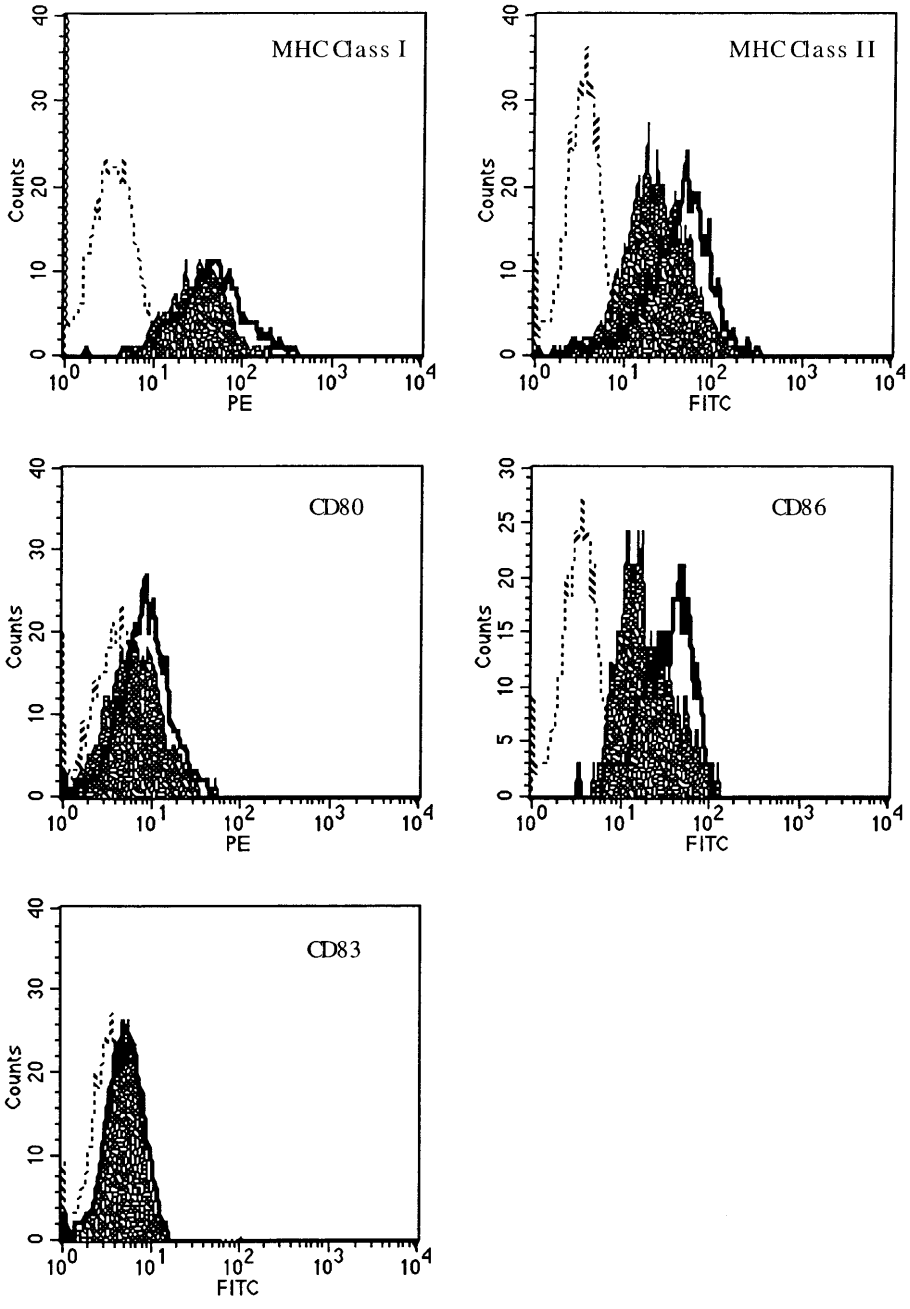

Fig. 1 Analysis of surface phenotypes of DCs and FCs in case 1. Both DCs and FCs were positive for MHC class I, class II and CD86, and weakly positive for CD80 and CD83. The expression of these surface markers on FCs was lower than those on DCs. *Dot histograms*, negative control; *open histograms*, level of fluorescence of surface phenotypes of DCs; *shaded histograms*, level of fluorescence of surface phenotypes of FCs

incubated not only with autologous glioma cells, but also with U87MG, suggesting that this response was not tumor-specific. In all cases, IFN-γ was not detected in the absence of restimulation with irradiated glioma cells.

Clinical responses

Vaccination with FCs was well tolerated in all patients and was administered on an outpatient basis. Vital signs monitored included blood pressure, temperature, pulse, cardiac rhythm and respiration. 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 (data not shown). In one case (case 6), erythema at the injection site was shown after the third immunization with FCs, suggesting that delayed-type hypersensitivity had occurred.

Clinical response data are listed in Table 2. There were two minor responses (cases 2 and 4). Case 2 suffered an uncontrollable headache despite the administration of analgesics before the immunization, which deteriorated after the immunizations. Interestingly, MRI showed that, although the size of the tumor itself did not change, the shift of the midline structure was improved and the high intensity area on the T2-weighted image was reduced (Fig. 3). Case 4 had hemiparesis that worsened at the beginning of immunization. However,

Table 2 Results of immunotherapy using fusion cells (*SD* stable disease, *PR* partial response, *PD* progressive disease, *DD* died of disease, *NC* no change, *MR* mixed reaction, *ND* not done)

Case	Initial vaccination	No. of vaccinations	Total amount of FCs ($\times 10^6$)	Fusion efficiency (%)	Clinical response after 8 weeks	Outcome in June 2000	Radiological findings	Adverse effects
1	August 1999	3	6.3	19.1	SD	DD	MR	No
2	September 1999	7	19.8	13.9	PR	PD	NC	No
3	December 1999	3	4.6	9.2	SD	PD	NC	No
4	December 1999	8	25.4	35.3	PR	PD	NC	No
5	February 2000	3	10.2	26.0	SD	PD	NC	No
6	March 2000	4	6.8	ND	SD	SD	NC	No
7	March 2000	1	12.0	9.9	PD	PD	NC	No
8	April 2000	2	9.9	ND	PD	DD	PD	No

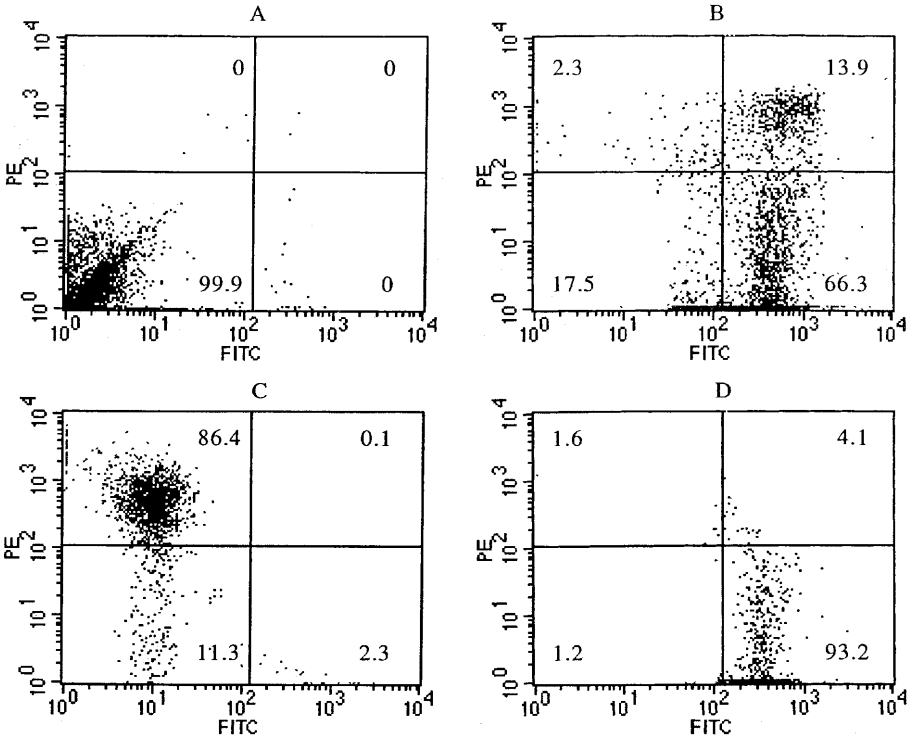

Fig. 2A–D Analysis of fusion efficiency using FACScan in case 2. A Negative control. B After staining dendritic and glioma cells with PKH 2 and PKH 26, respectively, both cells were fused with PEG. Double-positive cells (13.9%) were determined to be fusion cells. C PKH 26 was incorporated into glioma cells; 86.4% of glioma cells were positive for PKH 26. D PKH 2 was incorporated into DCs; 93.2% of DCs were positive for PKH 26. The *numbers* show the percentage of cells. *Vertical axis* PKH 26, *horizontal axis* PKH 2

the patient showed a slight improvement of hemiparesis after the immunizations, although MRI showed no change in the size of the tumor (data not shown). In both cases, symptoms improved within three weeks after the first immunization. In case 1, the size of primary tumor was decreased after the immunizations. However, the progression of a secondary lesion (Fig. 4) required surgical removal. Following culture, the expression of MHC class I and II was analyzed. No remarkable difference in the expression of MHC class I and II between primary and secondary tumor cells was found (data not shown). Then the difference in the response of PBLs against primary and secondary tumor cells was investigated. PBMCs were separated from blood taken before,

Table 3 Surface phenotypes of PBLs before and after immunization with fusion cells

Case	CD3 (%)		CD4 (%)		CD8 (%)		4/8 ratio		CD16 (%)		CD19 (%)		CD56 (%)	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
1	79.9	70.5	46.2	42.4	30.3	24.9	1.5	1.7	12.0	15.2	7.1	7.7	ND	19.9
2	61.6	63.5	53.8	56.2	10.3	12.5	5.2	4.5	5.6	5.9	29.7	27.6	5.2	8.4
4	75.9	68.4	40.3	37.7	33.8	31.3	1.2	1.2	10.4	13.2	6.5	15.7	13.8	15.3
5	85.1	79.4	42.4	40.2	46.4	45.8	0.9	0.9	10.3	11.8	2.5	5.8	28.2	35.4
6	82.2	82.4	47.1	52.7	34.8	31.5	1.4	1.7	10.0	7.9	7.3	7.7	15.6	14.2

Table 4 Concentration of IFN-γ produced by PBMCs before and after immunization with fusion cells. Results are presented as the mean ± SD (*Auto* autologous glioma cells, *Auto (P)* autologous glioma cells established from primary lesion, *Auto (S)* autologous glioma cells established from secondary lesion, *ND* not done)

Case	Target cells	IFN-γ concentration (pg/ml)	
		Before	After
1	U87MG	15.3 ± 2.1	17.6 ± 1.9
	Auto (P)	4.9 ± 0.8	8.7 ± 0.9
	Auto (S)	<4.0	<4.0
2	U87MG	<4.0	9.4 ± 0.6
	Auto	<4.0	8.3 ± 0.7
3	U87MG	8.5 ± 0.5	9.2 ± 0.5
	Auto	<4.0	6.7 ± 0.8
4	U87MG	72.5 ± 4.8	88.2 ± 5.7
	Auto	63.2 ± 2.5	73.9 ± 3.5
5	U87MG	241 ± 14.7	328 ± 29.1
	Auto	47.8 ± 6.3	89.8 ± 10.5
6	U87MG	113 ± 15.9	229 ± 26.1
	Auto	66.0 ± 5.3	219 ± 19.2
7	U87MG	ND	ND
	Auto	ND	ND
8	U87MG	ND	ND
	Auto	ND	ND

and 10 weeks after, the first immunization. PBMCs were incubated with irradiated primary or secondary glioma cells for 48 h and the supernatants were harvested. The concentration of IFN-γ was measured using ELISA. The concentration of IFN-γ in the supernatant of PBMCs incubated with primary glioma cells was high compared with that of PBMCs incubated with secondary glioma cells, suggesting that the patient's PBLs responded on primary glioma cells used as FCs, but not on secondary glioma cells (Table 4).

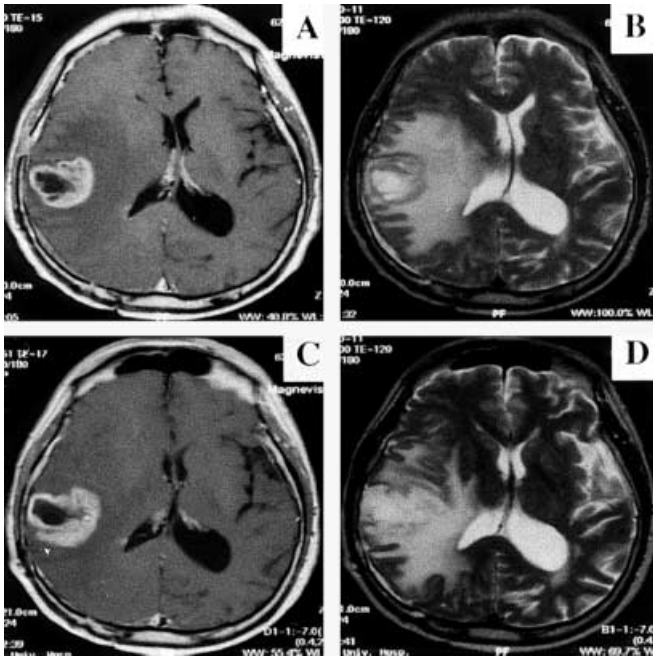

Fig. 3A–D MRI of case 2 shows that, although the size of the tumor itself did not change, the shift of the midline structure was improved due to a reduction of the brain edema. T1-weighted (A) and T2-weighted (B) images before immunization. T1-weighted (C) and T2-weighted (D) images after immunization

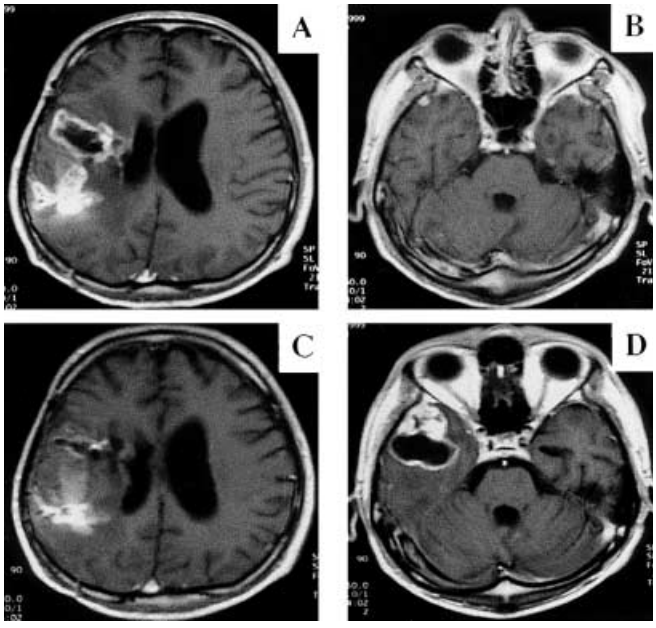

Fig. 4 T1-weighted MRI of case 1 after the immunizations shows that the size of the primary tumor was decreased (A, C) although there was progression of the secondary lesion (B, D). A, B Before immunization; C, D after immunization

Discussion

Genetically engineered glioma cells can be used as APCs for vaccination against gliomas, but the antitumor effect insufficiently eradicates established brain tumors in the mouse model [2, 30]. However, an intradermal injection of fusions with dendritic and glioma cells prolonged the survival of mice with brain tumors [1]. A DC-based vaccine is, therefore, a potential treatment for brain tumors [12, 20, 25]. Based on these experimental data, we started a clinical trial of immunotherapy using FCs.

The central nervous system (CNS) is generally considered an immunologically privileged site, due to the lack of lymphatic drainage and the nature of the blood-brain barrier (BBB), in which tight junctions between cerebral vascular endothelial cells form a physical barrier to the passage of cells and antibodies [7]. If this is the case, an immunotherapy may not be effective against brain tumors. However, pathological findings generally showed the existence of tumor-infiltrating lymphocytes in malignant gliomas, suggesting the potency of immunotherapy. In addition, several immunotherapies, including vaccination with genetically engineered glioma cells to express CD80 and CD54 and to produce IL-2 [15, 16, 17], and vaccination with FCs, prolonged the survival of tumor-bearing mice in our mouse brain tumor model. Therefore, the brain may not be completely

immuno-privileged or, alternatively, barriers to the immune system can be surmounted for certain tumors, resulting in crosstalk between systemic and focal immunity. In a circumstance such as this, immunological responses after immunization with FCs can be expected even in the CNS.

Mature DCs lose the ability to take up antigens. Therefore, use of mature DCs requires efficient methods to incorporate TAAs into DCs. Several methods using DCs for the induction of antitumor immunity have been investigated to date and some reports of the clinical application of immunotherapy using DCs have been published [14, 18, 22, 24]. DCs pulsed with tumor lysate or tumor peptides were generally used, whereas DCs fused with autologous tumor cells were used by Kugler et al. [18] and in our studies. It may be advantageous to use FCs as a potential therapeutic approach for malignant gliomas, since (1) the common TAAs of gliomas have not yet been identified, (2) FCs can be used to induce antitumor immunity against unknown TAAs, and (3) the induction of autoimmune responses against normal cells, including endothelial cells and neurons, can be avoided. On the other hand, the disadvantages are that (1) cultured glioma cells are needed, (2) irradiated glioma cells may still have tumorigenicity in vivo, (3) only glioma cells that adapt to the condition of the culture can be used, and (4) immune responses against normal glial cells may occur. A major difficulty for the future clinical development of this autologous treatment approach was the low yield of autologous glioma cells. Although it was not necessary to establish permanent cell lines, in some cases even primary cultured cells could not be obtained. Before beginning immunization, we investigated the tumorigenicity of irradiated autologous glioma cells. Irradiated tumor cells were incubated for two weeks to confirm that those cells did not grow. Kugler et al. reported the fusion of DCs with fresh tumor cells [18], whereas we fused DCs with cultured glioma cells. Our method avoids mixing normal cells. However, TAAs of recurrent tumors may not be the same as those of cultured tumor cells, resulting in the "escape phenomenon", in which CTLs induced by FCs can kill only tumor cells expressing the same TAAs as those of the cultured tumor cells. In case 1, after immunization with FCs, the size of primary tumor was decreased, while there was progression of the secondary lesion. We speculate that CTLs induced by administered FCs recognized only the primary, and not the secondary, tumors due to the difference in TAAs. Clinical trials with DCs have not reported an autoimmune response to date. It has been reported that in MUC1-transgenic mice, immunization with FCs with DCs and MUC1-positive tumor cells resulted in the rejection of established MUC1-positive tumor cells and no apparent autoimmunity against normal tissues [11]. On the other hand, Ludewig et al. reported that immunotherapy using peptide-pulsed DCs induced an autoimmune response in a mouse model [21]. Therefore, even if severe autoimmune responses against normal cells have not occurred in clinical trials, careful investigation is necessary.

DCs can sensitize $CD4^+$ T cells to specific antigens in an MHC-restricted manner. DCs produce interleukin 12 (IL-12), which acts as a growth factor for T and NK cells and induces production of IFN-γ from T and NK cells [3], implying that immunization with FCs induces activation of both T and NK cells. In the present study, surface phenotype analysis of PBMCs showed that the percentages of CD16- and CD56-positive cells were increased after immunization with FCs. In addition, the concentration of IFN-γ was high in supernatants of PBMCs cultured with either autologous or U87 glioma cells after immunization, compared with those before immunization. These results suggest that FCs activated mainly NK and/or Th1 cells. These results were not compatible with our data, using an animal model. In the mouse brain tumor model, antitumor effects of FCs were mediated via $CD8^+$ T cells [1].

The results of the phase I clinical trial of FCs with DCs and cultured autologous glioma cells indicated that this treatment safely induced immune responses. However, since our sample population was limited to 8 fully evaluable patients, we were unable to determine a statistically significant treatment-associated response rate. Our study in a mouse brain tumor model demonstrated that systemic administration of recombinant IL-12 enhanced the antitumor effects of FCs [1], suggesting that further evaluation of the role of adjuvant cytokines is necessary. IL-12, originally known as natural killer cell stimulatory factor or cytotoxic lymphocyte maturation factor, enhances the lytic activity of 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 [3]. These results indicate that IL-12 may be a potential candidate for an adjuvant cytokine. Based on data from this phase I trial, a phase II clinical trial of immunization with FCs and recombinant human IL-12 is currently under way.

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