Clinical Evaluation of Dendritic Cell Vaccination for Patients with Recurrent Glioma: Results of a Clinical Phase I/II Trial

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Abstract

Purpose: To investigate the safety and the immunologic and clinical responses of dendritic cell therapy for patients with recurrent malignant glioma.

Experimental Design: Twenty-four patients with recurrent malignant glioma (6 grade 3 and 18 grade 4 patients) were evaluated in a phase I/II clinical study of dendritic cell therapy. All patients were resistant to the standard maximum therapy. The patient's peripheral blood dendritic cells were generated with granulocyte macrophage colony-stimulating factor, plus interleukin 4 with or without OK-432, and pulsed with an autologous tumor lysate. Dendritic cells were injected intradermally, or both intratumorally and intradermally every 3 weeks.

Results: The protocols were well tolerated with only local redness and swelling at the injection site in several cases. Clinical responses were as follows: 1 patient with partial response, 3 patients with minor response, 10 patients with stable disease, and 10 patients with progressive disease. The patients whose dendritic cells were matured with OK-432 had longer survival times than the dendritic cells from patients without OK-432 maturation. The patients with both intratumoral and intradermal administrations had a longer survival time than the patients with intradermal administration only. Increased ELISPOT and delayed-type hypersensitivity responses after vaccination could provide good laboratory markers to predict the clinical outcome of patients receiving dendritic cell vaccination. The overall survival of patients with grade 4 glioma was 480 days, which was significantly better than that in the control group.

Conclusions: This study showed the safety and clinical response of autologous tumor lysate-pulsed dendritic cell therapy for patients with malignant glioma. Dendritic cell therapy is recommended for further clinical studies in malignant glioma patients.

Despite recent advances in radiation therapy, chemotherapy, and surgical resectioning, the prognosis for patients with malignant glioma is still very poor (1, 2). Therefore, the development of a new treatment modality is extremely important. Among the new treatments currently being investigated for malignant glioma, immunotherapy is theoretically very attractive because it offers the potential for high tumor-specific cytotoxicity (3–7). Dendritic cells are rare, hematopoietically derived leukocytes that form a cellular network involved in immune surveillance, antigen capture, and antigen presentation (8). The initial results obtained from clinical trials of dendritic cell–based therapy for B-cell lymphoma (9), melanoma (10), prostate cancer (11), and renal cell carcinoma (12) have recently been published, and the results are encouraging. Antigen-specific immunity was induced in the majority of patients during dendritic cell vaccination, and regression of metastases was observed in 30% of patients (10). There is an increasing number of reports regarding preclinical studies (13–18) and clinical studies (5–7, 19) demonstrating that systemic immunotherapy using dendritic cells is capable of inducing an antitumor response within the immunologically privileged brain, confirming that the central nervous system may not be an absolute barrier to dendritic cell–based immunotherapy. Early clinical studies with immunotherapy using tumor lysate pulsed dendritic cells suggest that this could be a promising strategy for patients with cancer (20–24) and malignant gliomas (6, 7, 25). In a previous report (6), we have described the vaccination of 10 malignant glioma patients with dendritic cells pulsed by an autologous tumor lysate. The safety and immunologic responses of the study were discussed. Here, we describe the clinical evaluation of 24 malignant glioma patients vaccinated with dendritic cells pulsed by an autologous tumor lysate.

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Received 1/16/05; revised 3/8/05; accepted 3/11/05.

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Patients and Methods

Patients. There were 24 patients enrolled in this phase I/II study: 8 women and 16 men with an age range from 20 to 80 years (average,

48.9 years; Table 1). Patients had histologically proven glioblastoma (grade 4), anaplastic astrocytoma, or other malignant gliomas (grade 3) according to the WHO criteria. After surgical resection of their tumor, patients had a course of external beam radiation therapy (standard dose, 40 Gy to the tumor with a 3-cm margin, 20 Gy boost to the whole brain) and nitrosourea-based chemotherapy. Patients were monitored for recurrence of their tumor during the initial and maintenance therapy by magnetic resonance imaging (MRI) or computed tomography, and had no chemotherapy or radiotherapy during the previous 4 weeks of dendritic cell therapy. The patients started receiving dendritic cell immunotherapy when recurrence was detected. Treatment was carried out at the Department of Neurosurgery, Niigata University Hospital. Twelve patients (cases 2, 4, 5, 7, 9, 11, 13, 14, 15, 17, 18, and 19 in Table 1) had a maintenance dose (predonine, 30 mg/d) of glucocorticoid therapy during the immunotherapy. The median Karnofsky performance scale was 62.5, ranging from 30 to 100. Exclusion criteria included pulmonary, cardiac, or other systemic diseases, acute infections, and a history of autoimmune disease.

Generation of dendritic cells. A concentrated 100 mL leukocyte fraction was generated through a 1-hour restricted peripheral blood leukapheresis processing 3 to 4 L of blood with each collection. Peripheral blood mononuclear cells (PBMC) were then purified using Ficoll-Hypaque (Sigma, Tokyo, Japan) density gradient centrifugation. PBMCs were resuspended in RPMI 1640 (Invitrogen, Tokyo, Japan) with 1% autologous heat-inactivated serum, plated at a concentration of 5×10^6 cells/mL, and allowed to adhere to 10 cm^2 dishes. Nonadherent cells were removed after 4 hours at 37°C in a humidified 5% CO_2/95% air incubator, and the adherent cells were cultured at 37°C for 7 days in RPMI 1640 supplemented with 1% heat-inactivated autologous serum in the presence of 1,000 units/mL recombinant human granulocyte macrophage colony-stimulating factor (Immunex Corp., Seattle, WA), 500 units/mL recombinant human interleukin 4 (R&D Systems, Inc., Minneapolis, MN), and 1% penicillin/streptomycin (Invitrogen).

Preparation of tumor lysate. Tumor tissue was removed from the vivid glioma portion seen in the microsurgical scope during operation, and immediately placed in PBS. Adjacent nonglioma tissue was removed using a scalpel, and tumor cells were dispersed to create a single-cell suspension. Aliquots were taken for cell counting and viability staining by trypan blue exclusion. Cells were lysed by three to five freeze cycles in liquid nitrogen and thaw cycles at room temperature. Lysis was monitored by light microscopy. Large particles were removed by centrifugation (15 minutes, $400 \times g$), and the supernatants were passed through a 0.45 μm filter. The protein content was determined and aliquots were stored at −80°C until use.

Dendritic cell pulsing. After 7 days of culture, semiadherent and nonadherent cells were harvested by pipetting and used as dendritic cells for pulsing with the tumor lysate as described below. In the phase I study, following 7 days of culture, the dendritic cells were cultured overnight with 50 μg/mL keyhole limpet hemocyanin (KLH; Calbiochem, Bad Soden, Germany) and the tumor lysate. In the phase II study, after 7 days of culture, half of the dendritic cells were cultured overnight with 50 μg/mL KLH and tumor lysate followed by 0.1 KE (KE: clinical unit)/mL penicillin-killed *Streptococcus pyogenes* (OK-432: Chugai Pharmaceuticals, Tokyo, Japan) for 24 hours. The remaining half of the dendritic cells were cultured overnight with 50 μg/mL KLH and prepared for intratumoral vaccination. Approximately 1×10^7 dendritic cells were cultured with 700 μg of autologous tumor lysate. The cells were washed thrice with PBS and resuspended in RPMI 1640 as described below.

Design of the phase I/II dendritic cell therapy trial. The study protocol was approved by the Ethical Committee of Niigata

Table 1. Patient characteristics

Case	Age/Gender	Pathologic diagnosis	KPS	Previous treatment
1	46/M	Glioblastoma	30	S, R, C
2	40/F	Glioma	40	S, R, C
3	53/F	Glioblastoma	80	S, R, C
4	47/F	Anaplastic mixed glioma	40	S, R, C
5	60/M	Glioblastoma	30	S, R, C
6	65/F	Glioblastoma	80	S, R, C
7	69/M	Glioblastoma	40	S, R, C
8	20/F	Glioblastoma	80	S, R, C
9	34/M	Anaplastic oligoastrocytoma	60	S, R, C
10	27/F	Glioblastoma	70	S, R, C
11	61/F	Glioblastoma	60	S, R, C
12	61/M	Glioblastoma	80	S, R, C
13	62/M	Glioblastoma	50	S, R
14	73/M	Anaplastic oligodendroglioma	50	S, R, C
15	39/M	Anaplastic astrocytoma	70	S, R, C
16	55/M	Glioblastoma	80	S, R, C
17	33/M	Glioblastoma	60	S, R, C
18	45/M	Anaplastic astrocytoma	60	S, R, C
19	53/M	Glioblastoma	40	S, R
20	42/M	Glioblastoma	90	S, R, C
21	44/F	Glioblastoma	70	S, R, C
22	80/M	Glioblastoma	100	S, R
23	37/M	Glioblastoma	70	S, R, C
24	28/M	Glioblastoma	70	S, R, C

Abbreviations: KPS, Karnofsky performance scale. S, surgery; R, radiation therapy; C, chemotherapy.

University. All patients provided informed consent before treatment. Dendritic cells were injected intradermally, close to a cervical lymph node, or intradermally and intratumorally, via an Ommaya reservoir. Those patients who had a settled Ommaya reservoir at an appropriate tumor cavity received an intratumoral dendritic cell injection. Patients were monitored for immediate and delayed toxicities. All toxicities were graded using the National Cancer Institute Common Toxicity Criteria. The response to the treatment was evaluated by clinical observations and radiological findings. MRI or computed tomography scanning was done to evaluate the intracranial lesions after the vaccinations every month. Tumor size was estimated as the volume of the region of abnormal enhancement observed on MRI or computed tomography via direct measurement. Responses were classified into the following categories based on the criteria of Macdonald et al. (26): (*a*) complete response, defined as a disappearance of the entire tumor for at least 4 weeks; (*b*) partial response, defined as a reduction of 50% or more in the tumor size for at least 4 weeks; (*c*) minor response, defined as a 25% to 50% decrease of the lesion lasting at least 4 weeks or a more than 50% decrease of the lesion lasting less than 4 weeks; (*d*) no change, defined as either a decrease of less than 25% or an increase of less than 25% in tumor size for at least 4 weeks; (*e*) progressive disease, defined as an increase of 25% or more in tumor size. The end points of this study were evaluations of the toxicity, immunologic response, and clinical response after dendritic cell therapy.

Delayed-type hypersensitivity reaction. To test the cell-mediated cytotoxicity response, 0.05 μg purified tuberculin and 10 μg autologous tumor lysate were intradermally administered into the forearm before and after treatment. A positive delayed-type hypersensitivity (DTH) skin test reaction was defined as >2 mm diameter induration after 48 hours.

IFN-γ ELISPOT assay. IFN-γ ELISPOT assay was done as previously described (6). Mononuclear cells (2×10^5/well) were used as stimulator cells, and tumor lysate-pulsed dendritic cells (1×10^4/well) were admixed to each well. A mouse antihuman IFN-γ antibody (clone 1-D1K; Mabtech, Nacka, Sweden), biotinylated detection antibody against human IFN-γ (clone 7-B6-1; Mabtech), and streptavidin-alkaline phosphatase complex preparation (Mabtech) were used. Spots were counted using a stereomicroscope (Zeiss, Jena, Germany) at ×40 magnification. The cutoff for positive spots was defined as a spot size greater than 3 × SD above the mean value of the spot diameter obtained in the absence of the dendritic cells.

Cell surface analysis. PBMCs were separated from the peripheral blood of patients, resuspended in PBS containing 1% bovine serum albumin (Sigma) and 0.1% sodium azide (Sigma), and stained with antihuman CD3, CD4, CD8, CD14, CD16, CD19, CD40, CD86, CD80, CD83, CD56, MHC I, and MHC II monoclonal antibodies (PharMingen, San Diego, CA) for 30 minutes at 4°C. Stained cells were washed and analyzed using FACScan (Becton Dickinson, San Jose, CA). Species- and isotype-matched monoclonal antibodies were used as controls.

Statistical analysis. The prevaccination and postvaccination data were compared using Wilcoxon's test. Statistical significance was determined at $P < 0.05$ level. The survival curves were estimated according to the method of Kaplan and Meier and the curves were compared using the generalized Wilcoxon's test. The log-rank test was used to assess the strength of the association between survival time and single variables corresponding to factors thought to be prognostic for survival. Survival was determined from the date of diagnosis to death or last visit.

Results

Isolation and characterization of dendritic cells. Mononuclear cells (0.35×10^8 – 10×10^8; average, 5.4×10^8) were isolated by Ficoll-Hypaque density gradient centrifugation and differentiated into dendritic cells in the presence of granulocyte macrophage colony-stimulating factor and interleukin 4. The final yield of dendritic cells after 7 days of culture was 0.3×10^8 to 6.2×10^8 (average, 1.54×10^8). Dendritic cells of the immature phenotype (CD3 negative, CD14 negative, CD16 negative/CD56 negative, CD19 negative, MHC I positive, MHC II positive, CD40 low, CD86 low, and CD83 low) were greater than 75% (data not shown). For seven patients, half of the dendritic cells were pulsed with tumor lysate and matured with OK-432, and the remaining half were stored without OK-432 and tumor lysate administration. With OK-432 administration, dendritic cells of the mature phenotype (CD80 positive, CD83 positive, and CD86 high) were greater than 80% (data not shown). These dendritic cells were divided into six tubes and cryopreserved in 80% RPMI 1640 supplemented with 10% autoplasma + 10% DMSO in liquid nitrogen. One cryopreserved vial was thawed and used for each vaccination. The dendritic cells were tested for endotoxin and *Mycoplasma* before administration to the patient.

Safety of dendritic cell therapy. Patients received the dendritic cells pulsed with the autologous tumor lysate every 3 weeks. The immunization was continued with up to 10 vaccinations, depending on the clinical response. Intradermal or both intratumoral and intradermal vaccinations with dendritic cells were done. Injections of 1×10^6 to 32×10^6 dendritic cell cells were used per vaccination. The mean number of administrations was 7.4 times intradermally and 4.6 times intratumorally, ranging from 1 to 22. The mean total number of inoculated dendritic cells was 5.318×10^7 cells for intradermal injections and 4.235×10^7 cells for intratumoral injections (Table 2). There were no serious adverse effects and no clinical or radiological evidence of autoimmune reactions in any of the patients (Table 2). There were no substantial changes in the results of routine blood tests (data not shown). Patient 6 developed a mild headache lasting a few days after vaccination. In seven cases (cases 4, 6, 12, 16, 20, 21, and 24), mild erythema at the cervical injection site was evident after the third immunization, suggesting that a DTH reaction had occurred.

Clinical responses. The clinical response data are listed in Table 2. There was one partial response (case 12: Fig. 1A and B), three minor response (cases 3, 6, and 24), and 10 cases with no changes on MRI. The MRI of case 12 shows that the contrast-enhanced lesion was decreased after vaccination. The MRI of case 22 (Fig. 1C and D) showed that the size of the contrast-enhanced area did not change for 1 year after vaccination.

Method of dendritic cell maturation and administration comparisons. In the phase I protocol ($n = 17$), immatured dendritic cells pulsed by tumor lysate were administered intradermally, or both intradermally and intratumorally. On the other hand, in the phase II protocol ($n = 7$), matured dendritic cells pulsed by tumor lysate were intradermally administered and immatured dendritic cells were intratumorally administered. In the phase I protocol, two minor response cases, six no change cases, and nine progressive disease cases were observed. On the other hand, in the phase II protocol, one partial response case, one minor response case, four no change cases, and one progressive disease case were obtained as evaluated by MRI. The patients with glioblastoma multiforme whose dendritic cells were matured with OK-432 ($n = 7$)

Table 2. Results of dendritic cell therapy

Case	No. vaccination	Total amount of dendritic cells (million)	Adjuvants	Radiological findings	DTH	ELISPOT	Adverse effects	Overall survival (d)	Outcome
1	2 (i.d.)	10 (id*)	KLH	NC†	Negative	n.d.‡	No	463	Dead
2	2 (i.d.), 2 (i.t.)	17 (i.d.), 17 (i.t.)	KLH	NC	n.d.	n.d.	No	1,526	Dead
3	4 (i.d.), 4 (i.t.)	42.4 (i.d.), 52.4 (i.t.)	KLH	MR	Positive	Negative	erythema (grade 1)	641	Dead
4	1 (i.d.), 1 (i.t.)	12.6 (i.d.), 12.6 (i.t.)	KLH	NC	n.d.	n.d.	No	410	Dead
5	2 (i.d.), 2 (i.t.)	64 (i.d.), 64 (i.t.)	KLH	PD	Negative	n.d.	No	466	Dead
6	10 (i.d.), 7 (i.t.)	137.18 (i.d.), 106.6 (i.t.)	KLH	MR	Positive	Positive	headache (grade 1)	1,466+	Alive
7	3 (i.d.)	46 (i.d.)	KLH	PD	n.d.	Negative	No	352	Dead
8	6 (i.d.)	37.5 (i.d.)	KLH	NC	Positive	Positive	No	417	Dead
9	4 (i.d.)	28.2 (i.d.)	KLH	PD	n.d.	Negative	No	649	Dead
10	5 (i.d.)	44.8 (i.d.)	KLH	PD	Negative	n.d.	No	472	Dead
11	4 (i.d.)	44.2 (i.d.)	KLH	PD	Negative	Negative	No	514	Dead
12	15 (i.d.)	138.8 (i.d.)	KLH/OK432	PR	Positive	Positive	erythema (grade 1)	735	Dead
13	10 (i.d.)	42.4 (i.d.)	KLH	PD	Negative	Negative	No	347	Dead
14	3 (i.d.), 3 (i.t.)	11.25 (i.d.), 1.25 (i.t.)	KLH	NC	n.d.	n.d.	No	305	Dead
15	6 (i.d.)	35.6 (i.d.)	KLH	PD	Negative	Negative	No	419	Dead
16	18 (i.d.), 18 (i.t.)	128.7 (i.d.), 139.1 (i.t.)	KLH/OK432	NC	Positive	Positive	erythema (grade 1)	630	Dead
17	2 (i.d.), 2 (i.t.)	5 (i.d.), 5 (i.t.)	KLH	PD	n.d.	n.d.	No	63	Dead
18	7 (i.d.), 7 (i.t.)	47.5 (i.d.), 47.5 (i.t.)	KLH	NC	Negative	Positive	No	698	Dead
19	6 (i.d.)	28.5 (i.d.)	KLH	PD	Negative	Negative	No	268	Dead
20	22 (i.d.), 3 (i.t.)	240.9 (i.d.), 13.5 (i.t.)	KLH/OK432	NC	Positive	Positive	erythema (grade 1)	1,172+	Alive
21	17 (i.d.), 2 (i.t.)	57.4 (i.d.), 7 (i.t.)	KLH/OK432	NC	Positive	Positive	erythema (grade 1)	864+	Alive
22	17 (i.d.)	37.22 (i.d.)	KLH/OK432	NC	Negative	Negative	No	333+	Alive
23	9 (i.d.)	15.3 (i.d.)	KLH/OK432	PD	n.d.	Negative	No	133+	Alive
24	3 (i.d.)	3.9 (i.d.)	KLH/OK432	MR	Positive	n.d.	erythema (grade 1)	186+	Alive

*i.d., intradermal vaccination; i.t., intratumoral vaccination.
†PR, partial response; MR, minor response; NC, no change; PD, progressive disease.
‡n.d., not tested.

had longer overall survival times ($P = 0.027$) than the patients without OK-432 maturation ($n = 11$; Fig. 2A). The glioblastoma multiforme patients with both intratumoral and intradermal administration ($n = 7$) had longer overall survival times ($P = 0.042$) than the patients with intradermal administration ($n = 11$; Fig. 2B).

Delayed-type hypersensitivity reactivity. DTH test using the autologous tumor lysate was done before and after treatment. Eight of 17 patients showed reactivity to the autologous tumor lysate; four of these had a partial or minor reaction and four had no change on MRI. Six patients had an increased ELISPOT result after vaccination. The glioblastoma multiforme patients ($n = 18$) with DTH response after vaccination had a significantly ($P = 0.003$) longer overall survival time than those without DTH response (Fig. 2C).

Detection of tumor lysate-reactive CD8+ T-cells in the blood of glioma patients correlates with longer survival. Blood samples of 16 glioma patients were tested by ELISPOT analysis for the presence of tumor lysate-reactive CD8+ T-cells in PBMC from patients before vaccination and 1 week after the last vaccination. To determine the frequency of tumor lysate-reactive CD8+ T-cells by the IFN-γ ELISPOT assay, monocytes were prepared from the blood of the 16 glioma patients, loaded with tumor lysate-pulsed dendritic cells, and then cocultured. As a negative control, PBMCs were not pulsed with dendritic cells. The results of the ELISPOT analysis are summarized in Fig. 3. T cells reactive against tumor lysate-pulsed dendritic cells were increased in seven patients after vaccination ($P < 0.05$). Two of these patients had a partial or minor reaction and five had no change on MRI. On the other hand, only a weak T-cell response against tumor lysate-pulsed dendritic cells was observed in nine glioma patients after vaccination. Also, the glioblastoma multiforme patients ($n = 18$) with ELISPOT response after vaccination had a significantly ($P = 0.015$) longer overall survival time than those without ELISPOT response (Fig. 2D).

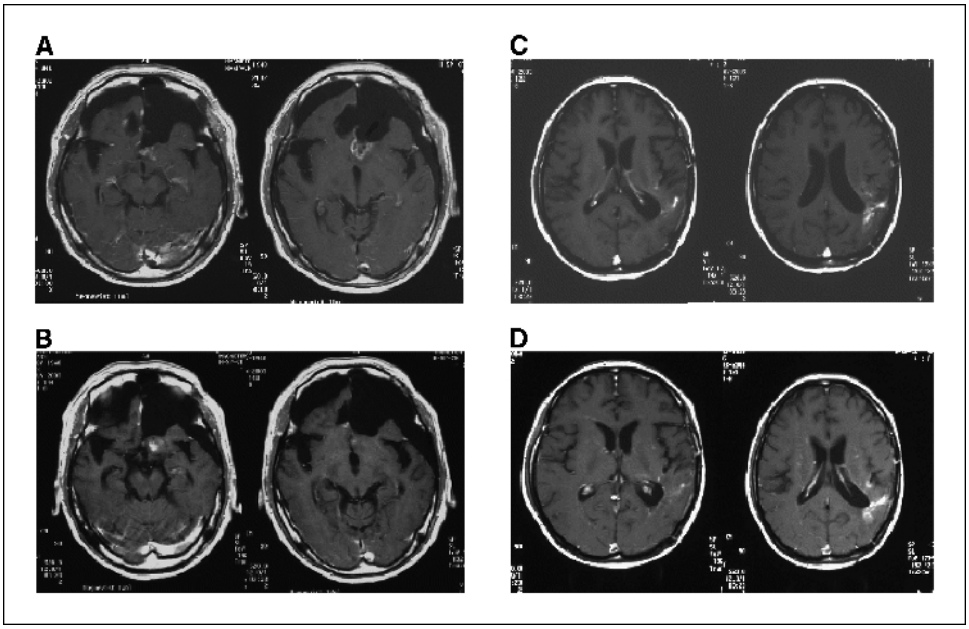

Fig. 1. MRI contrast-enhanced images of case 12 before (*A*) and after (*B*) vaccination showing that the fronto-basal lesion was decreased after vaccination. MRI contrast-enhanced images of case 22 before (*C*) and after (*D*) vaccination showing that the size of the contrast-enhanced left temporo-parietal area was stable for 1 year after vaccination.

Dendritic cell vaccination is associated with prolonged survival. To determine a survival benefit of dendritic cell vaccination in the patients, overall survival was assessed from 18 vaccinated patients and 27 nonselected controls from patients with glioblastoma multiforme. We compared the survival in our patient populations with age-, gender-, and disease-matched controls who had not received dendritic cell therapy. Control group patients underwent craniotomy, as the study group, and had completed external beam radiation therapy of 60 Gy and nitrosourea-based chemotherapy. They met all inclusion criteria for the dendritic cell immunotherapy trial. There were no statistically significant differences between the study and control groups for age (49.8 ± 16.1 versus 55.9 ± 11.9 years; $P = 0.151$) or percentage of patients with image complete resections (23.5% versus 25.9%; $P = 0.777$). Kaplan-Meier probability curves are shown in Fig. 4. The log-rank test revealed that the survival curves for the two groups were significantly different ($P = 0.010$; Fig. 4). In the study group, median overall survival time was 480 days (range, 63-1,466 days) whereas in the control group, median overall survival time was 400 days (range, 136-814 days). For the dendritic cell group, percentage of overall survival was 23.5% at 2 years. In the control group the percentage of overall survival was 3.7% at 2 years.

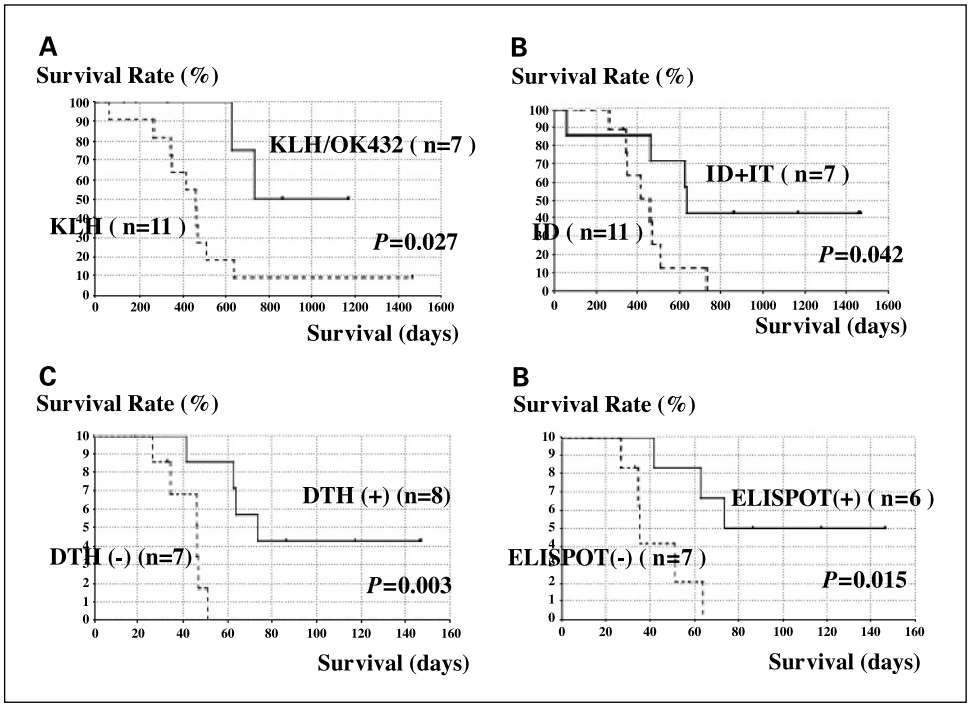

Fig. 2. Overall survival was compared between these two groups. Dendritic cells were cultured KLH with or without OK-432 administration and administered intradermally (*ID*) or both intratumorally and intradermally (*ID+IT*). The relevance of dendritic cell maturation (*A*) and administration route (*B*) in the overall survival time was analyzed. Patients were submitted to DTH tests (*C*) and ELISPOT (*D*) before and after vaccinations. Relevance of DTH and ELISPOT positivity in the overall survival time was also analyzed.

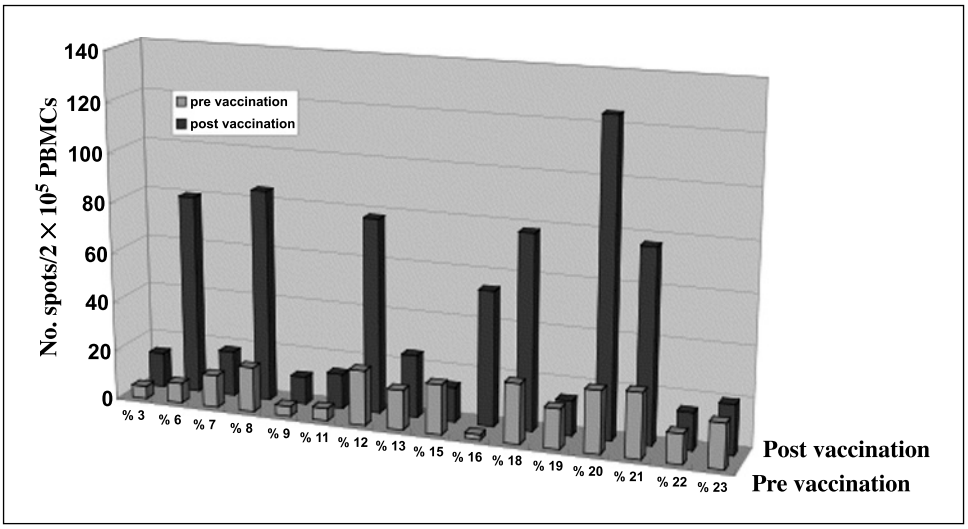

Fig. 3. T-cell – mediated antitumor activity of PBMCs of glioma patients as evaluated by ELISPOT assay before and after vaccination. Mean values of triplicate measurements were determined.

Discussion

The remarkable ability of dendritic cells to elicit an immune response and the availability of dendritic cell culture systems have allowed the use of dendritic cells in cancer immunotherapy. Large numbers of functional dendritic cells can be isolated from bone marrow precursor cells treated *in vitro* with cytokines such as granulocyte macrophage colony-stimulating factor, interleukin 4, and tumor necrosis factor α. Together, these findings show that autologous dendritic cells from tumor-bearing hosts can be expanded *ex vivo*, pulsed with tumor antigens, and reintroduced to induce tumor-specific T cells. In preclinical models and in clinical trials, these cytokine-stimulated dendritic cells have been successfully pulsed *ex vivo* with tumor antigens for use as antitumor vaccines against several types of malignancies.

The use of synthetic peptide approaches requires identification of tumor-specific antigens for individual tumors and demonstration of their recognition by CTLs, a process that is difficult. To date, therefore, there has been limited identification of the antigenic peptides and CTL epitopes presented by human gliomas (27). The advantages of vaccinating with total tumor-derived material, such as tumor cell lysates or tumor-derived mRNA, are that the identities of tumor antigens need not be known and that the use of multiple tumor antigens reduces the risk of antigen-negative escape mutants. There have been three reports using tumor-lysate pulsed dendritic cells for patients with recurrent gliomas (6, 7, 25). There were no serious adverse effects and no clinical or radiological evidence of autoimmune reactions in any of the patients in these studies except one patient who repetitively developed peritumoral edema (25). Although there is some probability of shared antigens between tumor and normal central nervous system tissues, and possible contamination of normal tissue in the tumor lysate, no autoimmunity was experienced *in vivo*. Furthermore, in our *in vitro* study, dendritic cells pulsed with a normal brain lysate failed to induce cytolytic T-cell activity against autologous glioma cells, suggesting the lack of an autoimmune response (28). The source of the dendritic cells in these cases was immature dendritic cells (6, 7) and mature dendritic cells (25). The administration route was intradermal injection (7, 25), and both intradermal and intratumoral injections (6). Dendritic cell vaccination elicited systemic cytotoxicity detected by IFN-γ expression in response to tumor lysate, and intratumoral cytotoxic T-cell infiltration was detected in several patients, although analysis of the function of these infiltrating cells should be carried out more precisely (6, 7, 25). Yu et al. (7) also reported prolonged median survival of 133 weeks in eight glioblastoma patients who received dendritic cell therapy.

Intratumorally injected dendritic cells would acquire and process tumor antigens *in situ*, migrate to regional lymphoid organs via lymphoid vessels, and initiate a significant tumor-specific immune response (29). It has been reported that dendritic cells have antigen capturing and processing as well as trafficking abilities, only during their immature phase (30). We generated immature-phase dendritic cells that were injected intratumorally in the phase I/II study. This study shows that intratumorally injected dendritic cells induce a more efficient antitumor immunoresponse. In all cases, the dendritic cell preparations were loaded with KLH protein because KLH has been shown to serve as a strong surrogate

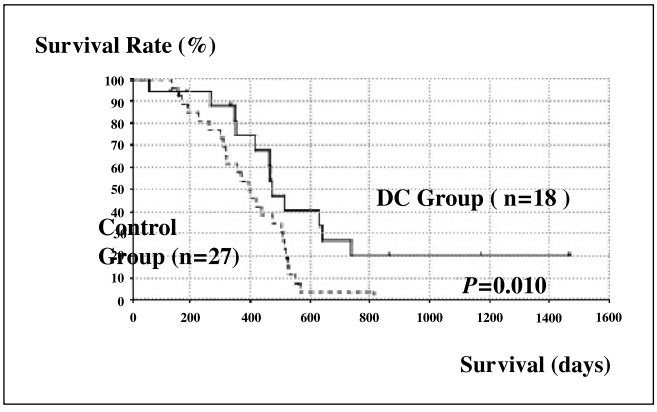

Fig. 4. Kaplan-Meier curves for overall survival time of the dendritic cell vaccination group ($n = 18$) compared with controls ($n = 27$).

antigen and an immunogenic marker for immunization studies using dendritic cell–based vaccines (9, 10). In the phase II study, we used mature dendritic cells, differentiated with OK-432, for intradermal vaccinations. Mature dendritic cells are reported to be superior to immature dendritic cells in the induction of antitumor immunologic responses (31, 32). We have experienced longer survival in patients with matured dendritic cell administration. The streptococcal preparation OK-432 is one of the biological response modifiers and has been clinically applied against various types of cancer because of its efficacy in enhancing the antitumor immune response (33, 34). OK-432 augments the cytotoxic activity of various effector cells such as lymphocytes, macrophages, and natural killer cells, and induces the production of multiple cytokines. OK-432 has the ability to induce the production of cytokines by dendritic cells and to promote the maturation of dendritic cells (35, 36). We also experienced increased cell surface expression of CD80, CD83, and CD86, mature phenotype markers of dendritic cells. Dendritic cells matured with OK-432 could be a likely candidate as an adjuvant for dendritic cell–based immunotherapy.

The present results also revealed that increased ELISPOT and DTH responses after vaccination could provide a good laboratory marker to predict the clinical outcome of glioma patients under the dendritic cell vaccination. Of significant importance was that patients treated with dendritic cell vaccinations showed markedly prolonged survival compared with controls who underwent conventional treatment. It should be noted that a compromised immune function is a common feature of advanced malignancy. DTH and ELISPOT responses after vaccination distinguished patients as to their overall survival time, confirming the association of poor immune function and disease progression. The fact that both ELISPOT and DTH responses showed an increase throughout the vaccination period indicates that the administration of dendritic cells has affected the patients' immune system.

Dendritic cell vaccination of patients with glioma seems to be safe and not associated with autoimmunity. In this study, toxicities included mild headache and erythema. Due to the small populations studied thus far, further evaluation of dendritic cell immunotherapy is necessary to determine the optimum dose of dendritic cells, the appropriate route of vaccination, the best source of tumor antigens, and methods of antigen loading. The dendritic cell–based immunotherapy strategy seems a promising approach for inducing an antitumor immune and a clinical response in patients with glioma. The efficacy of such protocols should be determined in randomized, controlled clinical trials. The development of methods for manipulating the vaccination of dendritic cells will enhance the clinical usefulness of dendritic cell–based biotherapy for malignant glioma.

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