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Laboratory Investigation

Tumor lysate and IL-18 loaded dendritic cells elicits Th1 response, tumor-specific CD8+ cytotoxic T cells in patients with malignant glioma

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Summary

In this study, we demonstrate that tumor lysate-loaded dendritic cells can elicit a specific CD8+ cytotoxic T lymphocyte response against autologous tumor cells in patients with malignant glioma. CTL from three of five patients expressed strong cytolytic activity against autologous glioma cells, did not lyse autologous lymphoblasts and were variably cytotoxic against the LAK-sensitive cell line Daudi. Also, DCs pulsed normal brain lysate failed to induce cytolytic activity against autologous glioma cells, suggesting the lack of autoimmune response. Two of five patients CD8+ T cells expressed a modest cytotoxicity against autologous glioma cells. CD8+ T cells isolated during these ineffective primings secreted large amounts of IL-10, less amounts of IFN-γ as detected by ELISA, Type 2 bias in the CD8+ T cell response accounts for the lack of cytotoxic effector function from these patients. Cytotoxicity against autologous glioma cells could be significantly inhibited by anti-HLA class I antibody. These data demonstrate that tumor lysate-loaded DC can be an effective tool in inducing glioma-specific CD8+ CTL able to kill autologous glioma cells *in vitro*. However, high levels of tumor specific tolerance in some patients may account for a significant barrier to therapeutic vaccination. Moreover, cytotoxic responses were augmented by transfecting DC with the gene for IL-18. For all five patients, CD8+T cells treated with IL18 transfected DC produced Th1 response. These results may have important implications for the treatment of malignant glioma patients with immunotherapy. DCs loaded with total tumor lysate and IL-18 may represent a method for inducing Th1 immunoresponses against the entire repertoire of glioma antigens.

Introduction

Despite advances in radiation and chemotherapy along with surgical resectioning, the prognosis for patients with malignant glioma is poor. Among the new treatments currently being investigated for malignant glioma, immunotherapy is theoretically very attractive, since it offers the potential for high tumor-specific cytotoxicity [1–3]. Active immunotherapy, intended to induce antigen-specific T cell responses to tumor antigens, has advanced as a concept for the treatment of glioma [3–9].

To date, there is limited presentation on the antigenic peptides and CTL epitopes presented by human tumors, with the exception of melanoma [10,11]. However, several reports have shown that multiple epitopes can be recognized by T cells on human tumors [12,13]. Therefore, an alternative strategy for effective vaccination of tumor patients may be the use of unfractionated tumor-derived materials such as whole tumor cells, peptides or lysates isolated from tumor cells. In this regard, effective tumor immunity in several murine glioma models has been induced using professional antigen presenting cells (APCs) such as dendritic cells (DCs) pulsed with unfractionated tumor-derived antigens in the form of peptides [8], cell lysates [4,5], cDNA [9] or messenger RNA (mRNA) [6]. Vaccination of mice with DCs loaded with antigens results in tumor-specific CTL responses capable of rejecting implanted tumors.

Several groups have recently established the significant role played by DC in the immune system and provide a rationale for using DC as adjuvants for human immunotherapy [14,15]. DCs are the most effective APCs at activating naive T cells [14,15], and recently the combination of GM-CSF and IL-4 has been shown to generate large number of DCs from peripheral blood monocyte precursors [16].

It is reported that murine and human DCs loaded with tumor lysate can stimulate CTL responses [4,5,17,18], and treatment of tumor-bearing mice with DCs pulsed with tumor lysate led to a significant reduction in tumor growth or survival benefit [4,5]. The advantage of loading DCs with tumor lysate is that lysate contain multiple epitopes that can bind to many HLA alleles. Tumor lysate-pulsed DCs may be used to stimulate T cell responses in patients without prior knowledge of their HLA haplotype.

IL-18 was initially identified as a cytokine that facilitates the production of IFN-γ induced by endotoxin [19]. IL-18 has many similarities with IL-1 (12% homology with IL-1α, and 19% with IL-1β, respectively). IL-18 is produced by cells of monocyte lineage, augments NK cell activity, and enhances proliferation of T cells [20]. IL-18 also promotes NK and T cells to secrete IFN-γ as well as GM-CSF [21]. IL-18 also enhances the production of Th1 type cytokines, which associated with antitumor cytotoxic T cell responses [21]. Delivering IL-18 gene to the site of tumor that are

genetically modified to express gene products that enhance their ability to elicit a response may bypass the requirement for selective recruitment and local activation, which are disordered within the tumor microenvironment [22]. Consistent with the notion that Th1 cells are involved in cancer immunology, administrations of IL-18 have significant antitumor effect [21]. Indicating that vaccination with IL-18 may be useful in inducing antitumor immunity.

The purpose of this study is to evaluate the hypothesis that DCs loaded with the tumor lysate of autologous glioma cells could stimulate a CTL response capable of lysing the autologous glioma cells *in vitro*. Here, we report the *in vitro* induction of HLA class I-restricted CD8+ CTLs in patients with malignant glioma. These results provide evidence that T cell responses specific for undefined tumor antigens can be generated in patients with glioma with tumor lysate-pulsed DCs. And also induction of HLA class I-restricted CD8+ CTLs is augmented by IL-18 gene transduction into DC. These results may have important implications for treatment with active or adoptive immunotherapy of malignant glioma.

Materials and methods

Patients

Five patients who had undergone subtotal removal for malignant glioma provided tumor tissue and peripheral blood mononuclear cells (PBMCs). Specimens were obtained at the time of surgery through the Neurosurgical Department at the Niigata University under approval of the Institutional Review Board. All patients had glioblastoma mutiforme according to the World Health Organization (WHO) criteria and average age, karnofsky perfomance status (KPS) were 58.6, 82 respectively (Table 1). Patients did not receive any form of therapy prior to surgery. Normal brain tissue was obtained from lobectomy for benign tumor surgery of 64 year old women.

Tumor cell lines

The lymphokine activated killer (LAK) sensitive target Daudi, baby hamster kidney (BHK) cell was purchased from Riken Cell Bank (Tsukuba, Ibaragi) and was maintained at 37 °C, 5% CO_2 in RPMI 1640 (Invitrogen, Tokyo), supplemented with 10% fetal bovine serum (FBS; Invitrogen). Fresh autologous tumor samples were obtained from surgical specimens from all patients and were reduced to single cell suspensions under sterile conditions at room temperature. Tumor tissue was mechanically disrupted and propagated in Matrigel basement membrane matrix (Becton Dickinson, Bedford, MA) in RPMI 1640 supplemented with 10% FBS, 2 mmol/l L-glutamine, 100 μg/ml streptomycin, and 100 U/ml penicillin. Fresh tumor cell lines were maintained initially in RPMI 1640, supplemented with 10% FBS at 37 °C, 5% CO_2. The cell suspension was then washed three times in RPMI and thereafter seeded in 75 cm^2 tissue cultures flasks (Corning, Cambridge, MA).

Table 1. Patient characteristics

Case	Age/sex	Pathological diagnosis	KPS
1	53, F	Glioblastoma multiforme	80
2	69, M	Glioblastoma multiforme	90
3	60, M	Glioblastoma multiforme	70
4	57, M	Glioblastoma multiforme	90
5	54, F	Glioblastoma multiforme	80

Tumor lysate preparation

10^8–10^9 autologous tumor cells cultured in RPMI 1640 10% FBS were washed three times with phosphate-buffered saline (PBS, pH 7.4) and harvested by scraping. Cells were lysed by three to five freeze cycles in liquid nitrogen and thaw cycles in room temperature. Lysis was monitored by light microscopy. Large particles were removed by centrifugation (15 min, $400 \times g$), supernatants were passed through a 0.45 μm filter, and stored at −80 °C until use. Protein concentration was then measured using a BCA Protein Assay Kit (Pierce, Rockford, IN). Aliquots of this lysate mixture were used to pulse DC.

Dendritic cells generation and pulsing by tumor lysate

A leukapheresis was performed on the patients and PBMCs were isolated over a Ficoll-Hypaque density gradient centrifugation. DCs were generated from PBMCs in the serum-free AIM-V medium (Invitrogen) as described previously [23]. Briefly, PBMCs were cultured in AIM-V medium. After 4 h at 37 °C, nonadherent cells were removed, and the adherent cells were cultured at 37 °C in a humidified 5% CO_2 incubator, in medium supplemented with human recombinant GM-CSF (rGM-CSF) ((1000 U/ml), Immunex, Seattle, WA) and IL-4 ((500 U/ml), Genzyme, Cambridge, MA). After 7 days of culture, DC were harvested for pulsing with tumor lysate as described below. No significant differences were noted in the expression of HLA-DR or co-stimulatory molecules (i.e., CD80, CD86), among DC cultures derived from these five patients (data not shown).

Tumor lysate in 250 μl Opti-MEM (Invitrogen) and the cationic lipid DOTAP (Boehringer Mannheim, Indianapolis, IN) in 250 μl Opti-MEM, were mixed in 12 × 75 mm polystyrene tubes (Falcon, Oxnard, CA) at room temperature for 30 min. The amount of tumor lysate used was 100 μg per 10^6 DCs. The complex was added to DCs (5×10^6 cells/ml) in Opti-MEM and incubated at 37 °C for 1 h. DCs were washed and cultured overnight at 37 °C in the presence of GM-CSF and IL-4 before use.

In vitro *induction of tumor-specific CTL responses*

Autologous PBMCs obtained (5×10^6 cells/ml) were co-cultured with antigen-pulsed DCs (1×10^4 cells/ml) in

the presence of IL-2 and IL-7 (10 U/ml, respectively; Genzyme). After 14 days, CD8+ T cells were isolated using CD8 microbeads (Miltenyi Biotech, Sunnyvale, CA) according to manufacturer's protocol. The purity of CD8+ cells was 95% or more by FACS analysis (data not shown). The CD8+ T cells were cultured in RPMI 1640 supplemented with 10% autoserum and 20 U/ml of IL-2 at 37 °C. Two days after purification, T cell were re-stimulated with antigen-pulsed DCs. The CTL:DC ratios was 100. CTL assays were done 10 days after re-stimulation.

Cytotoxic activity

A 6-h chromium (^{51}Cr) release assay was performed as previously described [9,23] to measure the cytotoxic reactivity of DC-tumor lysate stimulated CD8+ T lymphocytes. In addition to autologous tumor cells, Daudi cells were used as a target for the detection of LAK cell activity. Con-A activated peripheral blood lymphocytes were used as autologous control targets. To determine the structures on the effector and target cells involved in lysis, monoclonal antibodies (Mabs) were used to block cytotoxicity. ^{51}Cr-labelled tumor targets were pre-incubated with MAbs specific for HLA class I (W6/32; Dako, Carpinteria, CA) and its isotype control (IgG_1k mAb isotype standard; PharMingen). Cytotoxicity assays were also evaluated with purified CD8+ T cells 3 weeks after stimulation of T cell co-cultures with normal brain lysate-pulsed DC as described above.

Specific cytotoxic activity was determined using the formula: % specific release = ([experimental release – spontaneous release]/[total release – spontaneous release]) × 100. Spontaneous release was less than 10% of total release. Standard errors of the means of triplicate cultures were less than 5%.

As negative control targets, autologous lymphoblasts were prepared by 3-day stimulation with Con-A (Invitrogen; 1 g/ml) in RPMI-1640 plus rIL-2 (25 U/ml).

Enzyme-linked immunosorbent assay

Two-ml CD8+ T lymphocytes suspensions at a concentration of 1.0×10^6 ml^{-1} in RPMI complete medium supplemented with 5% autoserum were plated into six well plates and were cultured at 37 °C with 5% CO_2. After 48 h of incubation, the supernatants were sampled and frozen at −80 °C until use. Cytokine levels in the supernatant were determined by an enzyme-linked immunosorbent assay kits (Quantikine, R&D Systems, Minneapolis, IN) according to manufacturer's instructions. Sensitivity of the assay for IL-10, IFN-γ was 1.5 and 0.3 pg/ml, respectively. Samples and standards were tested in triplicate.

Phenotypic analysis of T cells and tumor cells

Enriched cultures of CD8+ T cells were phenotyped. Flow cytometry was performed using MAbs directly conjugated against the following human leukocyte antigens: FITC-anti-CD3, CD4, CD8, CD56 (NK cell), CD16 (B cell), CD25 (Regulatory T cell), HLA-DR (all Becton Dickinson, San Jose, CA) and analyzed on a FACScan (Becton Dickinson). Autologous tumor cells were analyzed for MHC class I expression. Isotype-matched controls (FITC-anti-IgG2a) from Becton-Dickinson was also used.

Cloning of human IL-18 and generation of GCsap -IL18 particles

The full-length human IL-18 cDNA was obtained from total RNA of spleen cells (Clontech, Tokyo) by reverse transcrtiption-polymerase chain reaction (RT-PCR) using a Perkin-Elmer RT-PCR kit (Perkin-Elmer, Norwalk, CT). The primers for the RT-PCR were: 5′-AGAGGAGACCATGGCTGCTGAACCA-GTAGAAGACAATTGC-3′, and 5′-GGGCTCGAG-CTATCTTCGTTTTGAACAGTGAACATTA TA-3′. The amplified cDNA was sequenced on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems Divisions, Perkin-Elmer, Foster City, CA) and contained the complete IL-18 coding region. The IL-18 cDNA was ligated into the plasmid GCsap [24] to form the expression vector GCsap-IL18. Retroviral particles were generated as described elsewhere [24]. Infectivity of recombinant viruses to BHK and DC was determined by transfer of the GCsap that can transduce the β-galactosidase gene.

Functional activity of IL-18

To test the functional activity of IL-18, 2×10^5 BHK cells were added to each well of a 24-well plate, which contained 1 ml of culture supernatant from 2×10^6 GCsap particles. The cells were incubated at 37 °C for 48 h. Quantitation of secreted IL-18 from transfected cell was carried out in an ELISA by using IL-18 ELISA kit (PharMingen, San Diego, CA).

Results

HLA class I expression by tumor cells

MHC class I expression was investigated by FACS analysis on the tumor cell lines established from all five patients. All tumor cell lines expressed MHC class I molecules (data not shown).

Cytokine production of retroviral IL-18 transduced DCs

DCs were transfected with GCsap-LacZ using these transfection methods and incubated for 24 h. Transfected DCs were then analyzed by X gal staining. The transfection efficiency was 25 ± 8% at multiplicity of infection (MOI) of 100. Retroviral IL-18 transduced DCs produced significant quantities of IL-18. In contrast, the culture medium of both GCsap-LacZ transduced DCs and nontransduced DCs did not secrete detectable levels of IL-18 (Table 2).

Table 2. DCs can be efficiently transduced with an GCsap-IL18 vector and produce IL-18

	GCsap-LacZ	GCsap-IL18
24 h	<10	580 ± 120
48 h	<10	880 ± 400

DCs were transduced with GCsap-LacZ and GCsap-IL18, respectively. After transduction, supernatant in culture was collected and analyzed using an IL-18 ELISA. Accumulation of IL-18 was observed.

Tumor-specific CD8+ cytotoxic T cell responses

Cytotoxicity assays were evaluated with purified CD8+ T cells 3 weeks after stimulation of T cell co-cultures with tumor lysate-pulsed DC. Results are presented as the mean values from at least three independently primed CD8+ T cell cultures from each patient. As shown in Figure 1, cytotoxicity against autologous tumor cells was demonstrated for five patients at an effector:target ratio 50, 25, 12.5, 6.25: 1. For these patients, tumor-specific cytotoxicity was significantly inhibited by blocking MAb against HLA class I (significant at $P < 0.01$). Lysis of LAK-sensitive Daudi cells was also observed, notably for the CD8+ T cells from these five patients. However, lysis of autologous tumor cells was significantly higher than lysis of Daudi cells ($P < 0.01$ for patients 1, 2 and 5). These results suggest that specific, HLA class I-restricted cytotoxicity against autologous tumor antigens is a major component of the CD8+ T cell response following stimulation with tumor lysate-pulsed DC, although an LAK-like CD8+ cytotoxic T cell response is also detected from these patients. In contrast to the cytotoxic responses generated from patients 1, 2 and 5, we were able to generate only a very low level of T cell mediated cytotoxicity from a single DC-primed CD8+ T cell culture from patient 3 and 4 (Figure 1). However, T cell mediated cytotoxicity from these two patients was augmented by IL-18 gene transfection on DC. These results demonstrate that IL-18 gene transfer may enhance the therapeutic effects of DC-based therapy against malignant glioma. However, T cell mediated cytotoxicity from five patients was not augmented by IL-18 gene transfection on DC without tumor lysate. For all five patients, minimal levels of cytotoxicity against autologous Con-A-stimulated lymphoblasts were observed. Also, for all five patients, minimal levels of cytotoxicity against autologous tumor cells with purified CD8+ T cells 3 weeks after stimulation of T cell co-cultures with normal brain lysate-pulsed DC were observed. Cytotoxicity against autologous tumor cells was demonstrated for five patients at an effector:target ratio 50:1 as 8 ± 5%.

Phenotypic analysis of CD8+ T cells

Flow cytometric analysis was used to determine the phenotype of the populations of tumor-stimulated CD8+ T cells derived from the five patients. All the cells were CD3/CD8+ and CD4-, with a variable proportion of CD56 antigen positive cells. CD8+ T cells were also CD25+ and HLA-DR+ (data not shown).

Cytokine production

In unstimulated cultures, the production of IL-10 from CD8+ T cells was considerably increased in two cases (case 3 and 4), compared to the other three cases (case 1, 2 and 5) ($P < 0.01$) (Table 3).

A basal decrease of the production IFN-γ in case 3 and 4, compared to case 1, 2 and 5 was noted ($P < 0.01$). The increase of IFN-γ levels with IL-18 gene transfection leads to similar levels among these five patients (Table 3).

Then in the absence of IL-18 gene transduction, there is a definite activation of T lymphocytes of case 3 and 4 toward Th2 phenotype.

Discussion

To date, the possible immunization of cancer patients using defined tumor antigens is limited at present to

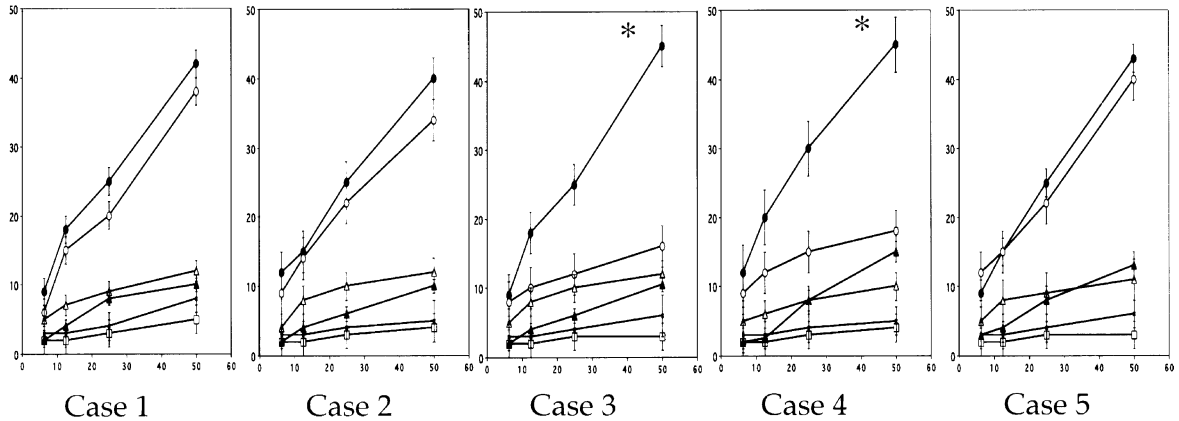

Figure 1. Tumor-specific CD8+ CTL responses induced by tumor lysate-pulsed and with/without IL-18 gene transfected DCs in patients with glioma, measured in a 6 h ^{51}Cr-release assay. Percentage lysis (±standard deviation) at a 50, 25, 12.5, 6.25 : 1 effector/target cell ratio is shown. Anti-HLA class I blocking antibody (W6/32) was used at 50 μg/ml. Targets are, autologous tumor cell cultured with IL-18 transfected and tumor lysate pulsed DC (●); autologous tumor cell cultured with tumor lysate pused, without IL-18 transfected DC (○); autologous tumor cell cultured with IL-18 transfected, without tumor lysate pused DC (▲); Daudi (△); autologous lymphoblast (□); autologous tumor+W6/32 anti-class I Mab (×). Values are presented as the mean of three independently primed T cell cultures. *$P < 0.01$.

Table 3. IFN-γ and IL-10 production by tumor-specific CD8+ T cells after stimulation with DC pulsed with/without IL-18 transduction

Cytokine	Case 1	Case 2	Case 3	Case 4	Case 5
(*A*) *Without IL-18 gene transfection*					
IFN-γ	10.0 (±3.0)	12.5 (±2.5)	1.0 (±1.0)*	2.5 (±2.0)*	16.0 (±4.0)
IL-10	10.0 (±4.0)	18.0 (±6.5)	90.0 (±16.0)*	120.5 (±25.0)*	20.8 (±6.0)
(*B*) *With IL-18 gene transfection*					
IFN-γ	540.2 (±150.0)	600.5 (±200.0)	400.0 (±180.0)	360.0 (±100.0)	45.0 (±250.0)

Results, given as means (±standard deviation), are expressed in pg/ml. *$P < 0.01$

some of human tumor types in which candidates for tumor antigens have been identified. Despite the elucidation of a number of defined tumor antigens from human tumors, a recent study in patients with melanoma revealed that most of the T cells specific for the tumor did not recognize well-known antigens, suggesting that the tumor antigens may vary from patient to patient or are undefined [25]. In an attempt to overcome these major obstacles, several investigators have recently reported induction of effective tumor immune response using DCs pulsed with unfractionated tumor-derived materials, such as acid eluted peptides, tumor lysate or messenger RNA (mRNA) [26–28]. DC vaccine treatment might be extended to patients with a variety tumors, provided that *in vitro* confirmation of tumor-specific CTL activity has been achieved.

In this study, we have shown that strong CD8+ cytotoxic T cell responses against autologous glioma cells can be elicited in patients with malignant glioma following stimulation of PBMCs with glioma lysate-pulsed DC. Although CD8+ cytotoxic T cells stimulated in this fashion showed some lytic activity against LAK-sensitive Daudi cells, much higher levels of cytotoxicity against autologous glioma cells were observed from three of five these patients. Furthermore, tumor-specific cytotoxicity was significantly inhibited by blocking MAb specific for HLA class I, indicating that a HLA-restricted CD8+ cytotoxic T cell response against glioma antigens was a major component of the response. Autologous Con-A activated blasts were not killed by glioma specific CTLs, indicating that while these CTLs were highly cytolytic for autologous glioma cells, they failed to kill autologous normal cells. Also DCs pulsed normal brain lysate failed to induce cytolytic activity against autologous glioma cells, suggesting the lack of autoimmune response. Unfractionated glioma lysate-loaded DCs elicit responses to a host of yet unidentified glioma antigens. Immunization with DC pulsed with autologous glioma lysate may provide a strategy to induce glioma-specific T cells from patients against a broad repertoire of glioma antigen, without the need to know the antigenic profile of each patient.

T cell-mediated protection against tumors is thought to be promoted by Type 1 cytokine responses and impaired by Type 2 cytokine responses [29]. In general, Type 1 T cells express IL-2, IFN-γ and TNF-β and favor cell-mediated immunity, delayed-type hypersensitivity, macrophage activation and the production of opsonizing antibodies and are cytotoxic, whereas Type 2 T cells express IL-4, IL-5, IL-6, IL-10 and IL-13, and favor humoral responses, providing for antibody production, and promote both mast cell growth and eosinophil differentiation and activation, resulting in humoral responses, and are noncytotoxic. Consistent with this notion, recent studies have showed significant dysfunction of Type 1 T cell responses in patients with cancer, suggesting that progression of disease may be associated with a preferential Type 2 T cell response [29,30]. The reasons why glioma lysate-pulsed DC stimulated CD8+ T cells from these primings for patient 3 and 4 were not able to generate a cytotoxic response against autologous glioma cells are open to discussion. We consider that a strong Type 2 bias in the CD8+ T cell response accounts for the lack of cytotoxic effector function from these patients. Supporting this idea, CD8+ T cells isolated during these ineffective primings secreted large amounts of IL-10, less amount of IFN-γ as detected by ELISA. There is a basal decrease of Th1 cytokine and an increase of Th2 ones. Extensive glioma progression may thus be associated with tumor-specific tolerance.

IL-18 was identified as a novel cytokine that induces IFN-γ secretion from NK and T cells, was initially termed IFN-γ inducing factor [20]. IL-18 augments NK cytolytic activity and enhances proliferation of T cells [20], activate CD8+ cytotoxic T lymphocytes [31]. These observations are consistent with *in vitro* studies that demonstrate that IL-18 co-operates with IL-12 to enhance production of Th1 type cytokines [31] and proliferation of Th1 cells [31]. Although all cells were treated in the same way, cells from different patients had different cytokine profiles, indicating different T cell subsets or differentiation pathways had been stimulated. In case 3 and 4, tumor-specific CTL responses induced by tumor lysate-pulsed DC was suppressed, possibly by up-regulation of IL-10 production and down-regulation of IFN-γ production. Induction of HLA class I-restricted CD8+ CTLs is augmented by IL-18 gene transduction into DC. IL-18 induced phenotypic changes could be due to the direct effects of IL-18 and/or indirect effects such as promoted via IFN-γ production from IL-18 activated T cells. We have demonstrated that IL-18 transfection of DCs enhances their immunostimulatory function for T cell activation.

Taken together, the findings of this study show that patients carrying locally advanced glioma can elicit a powerful cytotoxic T cell response against their autologous glioma *in vitro* following glioma lysate-loaded DC stimulation. However, evidence of glioma specific tolerance in some patients may account for a significant barrier to DC-based immunotherapy. In

this group of patients, attempts to overcome established Th2 responses with the use of strong Th1 agents such as IL-18 might have therapeutic implications. These results suggest that IL-18 may be useful to generate autologous CTL in glioma patients and may contribute to adoptive immunotherapy for malignant glioma.

References

1. Dranoff G, Jaffee E, Lazenby A, Golumbek P, Levitsky H, Brose K, Jackson V, Hamada H, Pardoll D, Mulligan RC: Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony-stimulating factor stimulates potent, specific, and long-lasting anti-tumor immunity. Proc Natl Acad Sci USA 90: 3539–3543, 1993
2. Sampson JH, Archer GE, Ashley DM, Fuchs HE, Hale LP, Dranoff G, Bigner DD: Subcutaneous vaccination with irradiated, cytokine-producing tumor cells stimulates CD8+ cell-mediated immunity against tumors located in the "immunologically privileged" central nervous system. Proc Natl Acad Sci USA 93: 10399–10404, 1996
3. Yu JS, Wheeler CJ, Zeltzer PM, Ying H, Finger DN, Lee PK, Yong WH, Incardona F, Thompson RC, Riedinger MS, Zhang W, Prins RM, Black KL: Vaccination of malignant glioma patients with peptide-pulsed dendritic cells elicits systemic cytotoxicity and intracranial T cell infiltration. Cancer Res 61: 842–847, 2001
4. Aoki H, Mizuno M, Natsume A, Tsugawa T, Tsujimura K, Takahashi T, Yoshida J: Dendritic cells pulsed with tumor extract-cationic liposome complex increase the induction of cytotoxic T lymphocytes in mouse brain tumor. Cancer Immunol Immunother 50: 463–468, 2001
5. Heimberger AB, Crotty LE, Archer GE, McLendon RE, Friedman A, Dranoff G, Bigner DD, Sampson JH: Bone marrow-derived dendritic cells pulsed with tumor homogenate induce immunity against syngeneic intracerebral glioma. J Neuroimmunol 103: 16–25, 2000
6. Insug O, Ku G, Ertl HC, Blaszczyk-Thurin M: A dendritic cell vaccine induces protective immunity to intracranial growth of glioma. Anticancer Res 22: 613–621, 2002
7. Kikuchi T, Akasaki Y, Irie M, Homma S, Abe T, Ohno T: Results of a phase I clinical trial of vaccination of glioma patients with fusions of dendritic and glioma cells. Cancer Immunol Immunother 50: 337–344, 2001
8. Liau LM, Black KL, Prins RM, Sykes SN, DiPatre PL, Cloughesy TF, Becker DP, Bronstein JM: Treatment of intracranial gliomas with bone marrow-derived dendritic cells pulsed with tumor antigens. J Neurosurg 90: 1115–1124, 1999
9. Yamanaka R, Zullo SA, Tanaka R, Blaese M, Xanthopoulos KG: Enhancement of antitumor immune response in glioma models in mice by genetically modified dendritic cells pulsed with Semliki forest virus-mediated complementary DNA. J Neurosurg 94: 474–481, 2001
10. Castelli C, Storkus WJ, Maeurer MJ, Martin DM, Huang EC, Pramanik BN, Nagabhushan TL, Parmiani G, Lotze MT: Mass spectrometric identification of a naturally processed melanoma peptide recognized by CD8+ T cells. J Exp Med 181: 363–368, 1995
11. Cox AL, Skipper J, Chen Y, Henderson RA, Darrow TL, Shabanowitz J, Engelhard VH, Hunt DF, Slingluff CL: Identification of a peptide recognized by five melanoma-specific human cytotoxic T cell lines. Science 264: 716–719, 1994
12. Peoples GE, Goedegeboure PS, Smith R, Linehan DC, Yoshino I, Eberlein TJ: Breast and ovarian-cancer specific cytotoxic T lymphocytes recognize the same HER/2neu-derived peptide. Proc Natl Acad Sci USA 92: 432–436, 1995
13. Yoshino I, Goedegeboure PS, Peoples GE, Parikh AS, DiMain JM, Lyerly HK, Gazdar AF, Eberlein TJ: HER2/neu-derived peptides are shared antigens among human non-small cell lung cancer and ovarian cancer. Cancer Res 54: 3387–3390, 1994
14. Steinman RM: The dendritic cell system and its role in immunogenicity. Ann Rev Immunol 9: 271–296,1991
15. Young JW, Inaba K: Dendritic cells as adjuvants for class I major histocompatibility complex-restricted antitumor immunity. J Exp Med 183: 7–11, 1996
16. Romani N, Gruner S, Brang D, Kampgen E, Lenz A, Trockebacher B, Konwalinka GO, Fritsch P, Steinman RM, Schuler G: Proliferating dendritic cell progenitors in human blood. J Exp Med 180: 83–93, 1994
17. Yu JS, Wheeler CJ, Zeltzer PM, Ying H, Lee PK, Incardona F, Yong WH, Thompson RC, Riedinger MS, Black KL: Dendritic cell immunotherapy for patients with glioblastoma multiforme and anaplastic astrocytoma. Proc Am Assoc Cancer Res Abstr 42: 274, 2001
18. Yamanaka R, Abe T, Yajima N, Tsuchiya N, Homma J, Kobayashi T, Narita M, Takahashi M, Tanaka R: Vaccination of recurrent glioma patients with tumor-lysate pulsed dendritic cells elicits immune responses: results of clinical phase I/II trial. Br J Cancer 89: 1172–1179, 2003
19. Nastala CL, Edington HD, McKinney TG, Tahara H, Nalesnik MA, Brunda MJ, Gately MK, Wolf SF, Schreiber RD, Storkus WJ: Recombinant IL-12 administration induces tumor regression in association with IFN- production. J Immunol 153: 1697–1706, 1994
20. Ossendorp F, Mengede E, Camps M, Filius R, Melief CJ: Specific T helper cell requirement for optimal induction of cytotoxic T lymphocytes against major histocompatibility complex class II negative tumors. J Exp Med 187: 693–702, 1998
21. Micallef MJ, Yoshida K, Kawai S, Hanaya T, Kohno K, Arai S, Tanimoto T, Torigoe K, Fujii M, Ikeda M, Kurimoto M: In vivo antitumor effects of murine interferon–inducing factor/interleukin-18 in mice bearing syngeneic Meth A sarcoma malignant ascites. Cancer Immunol Immunother 43: 361–367, 1997
22. Yamanaka R, Yajima N, Abe T, Tsuchiya N, Homma J, Narita M, Takahashi M, Tanaka R: Dendritic cell-based glioma immunotherapy (Review). Int J Oncol 23: 5–15, 2003
23. Kobayashi T, Yamanaka R, Homma J, Tsuchiya N, Yajima N, Yoshida S, Tanaka R: Tumor mRNA-loaded dendritic cells elicit tumor-specific CD8(+) cytotoxic T cells in patients with malignant glioma. Cancer Immunol Immunother 52: 632–637, 2003
24. Onodera M, Yachie A, Nelson DM, Welchlin H, Morgan RA, Blaese RM: A simple and reliable method for screening retroviral producer clones without selectable markers. Hum Gene Ther 8: 1189–1194, 1997
25. Anichini A, Mortarini R, Maccalli C, Squarcina P, Fleischhauer K, Mascheroni L, Parmiani G: Cytotoxic T cells directed to tumor antigens not expressed on normal melanocytes dominate HLA-A2.1-restricted immune repertoire to melanoma. J Immunol 156: 208–217, 1996
26. Boczkowski D, Nair SK, Snyder D, Gilboa E: Dendritic cells pulsed with RNA are potent antigen presenting in vitro and in vivo. J Exp Med 184: 465–472, 1996
27. Nair SK, Snyder D, Rouse BT, Gilboa E: Regression of tumors in mice vaccinated with professional antigen-presenting cells pulsed with tumor extracts. Int J Cancer 70: 706–715, 1997
28. Zitvogel L, Majordomo JI, Tjandrawan T, DeLeo AB, Clarke MR, Lotze MT, Storkus WJ: Therapy of murine tumors with tumor peptide-pulsed dendritic cells: dependence on T cells, B7 costimulation, and T helper cell 1-associated cytokines. J Exp Med 183: 87–97, 1996
29. Romagnani S: Human TH1 and TH2 subsets: regulation of differentiation and role in protection and immunopathology. Int Arch Allergy Immunol 98: 279–285, 1992
30. Ghosh P, Komschlies KL, Cippitelli M, Longo DL, Subleski J, Ye J, Sica A, Young HA, Wiltrout RH, Ochoa AC: Gradual loss of T-helper 1 populations in spleen of mice during progressive tumor growth. J Natl Cancer Inst 87: 1478–1483, 1995

31. Kohyama M, Saijyo K, Hayasida M, Yasugi T, Kurimoto M, Ohno T: Direct activation of human CD8+ cytotoxic T lymphocytes by interleukin-18. Jpn J Cancer Res 89: 1041–1046, 1998

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