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Review Article

Dendritic Cell Therapy of Primary Brain Tumors

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Abstract

Background: Although current treatment modalities for malignant gliomas, such as surgery, radiation and chemotherapy, have been improved markedly in the past two decades, the prognosis of these neoplasms remains poor, the two year survival rate being approximately 5%. Therefore, alternative treatment options, such as gene therapy and immunotherapy are rapidly gaining momentum. One of the most promising immunotherapeutic approaches for the treatment of cancer is the vaccination of cancer patients with dendritic cells (DC) pulsed with tumor antigens. Immunotherapy with DC seems to be able to overcome, at least partially, the immunosuppressive state associated with primary malignant gliomas. DC therapy proved to be safe in both animal models and clinical trials. No serious side effects and no evidence of autoimmune toxicity occurred. Most studies used DC pulsed with an array of tumor-associated antigens rather than single peptides, to allow for presentation of unknown tumor-specific antigens to DC. Routes of administration either were subcutaneous, intradermal or intraperitoneal, with multiple injections of DC to enhance antitumor immunity. DC therapy as an adjuvant treatment for patients with malignant glioma seems to be biologically safe. Further clinical studies are warranted.

Dendritic Cell Biology

The central nervous system (CNS) has long been thought to represent an immunologically privileged site, due to existence of the blood brain barrier, the absence of lymphatic vessels and dendritic cells (DC) in the CNS parenchyma, and the presence of an immunosuppressive microenvironment. However, it is now recognized that under pathological conditions, such as inflammation or tumor growth, lymphocytes may infiltrate the brain and a systemic immune response may be elicited (1,2).

Although tumor cells can present antigens via the MHC class I pathway, they are known to only weakly stimulate CTL responses *in vivo*. Possible explanations for this phenomenon include inefficient antigen presentation, lack of costimulatory molecules needed for priming of T cells and secretion of immunosuppressive factors such as TGF-β and IL-10, rendering tumor-infiltrating CTL anergic and tolerant (3). Therefore, professional antigen-presenting DC pulsed with tumor antigens are increasingly being explored in cancer vaccination studies. Dendritic cells are the most potent antigen presenting cells (APC) known to date. Besides being central inductors of immunity they may also play a critical role in maintaining self-tolerance (4). Upon stimulation by inflammatory mediators and pathogens immature DC residing in peripheral tissues efficiently uptake and process antigens, migrate to the T cell areas of lymphoid organs, and mature. Mature DC lose their antigen-capturing capacity and present processed endogenous and exogenous antigens to naïve T cells in an HLA-restricted manner. Besides expressing high levels of both MHC class I and class II molecules, concomitant expression of adhesion and costimulatory molecules, such as CD40, CD54 (ICAM-1), CD80 (B7-1) and CD86 (B7-2) as well as secretion of stimulatory cytokines such as IL-12, IL-15, IL-18 leads to efficient priming of cytotoxic T cells (CTL) and $CD4^+$ T helper cells. This results in the induction of a specific protective and therapeutic anti-tumor immune response, as demonstrated in several animal models of cancer (5). CTL are thought to be the main effector cells in tumor rejection. These cells recognize antigens loaded onto

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MHC class I molecules, which predominantly present peptides derived from endogenous antigens, while MHC class II-restricted presentation of exogenous antigens by DC leads to $CD4^+$ T cell activation. Recent findings suggest that DC can also prime $CD8^+$ T cell-mediated responses against exogenous tumor-associated antigens by cross-presenting peptides derived from engulfed apoptotic tumor cells (6).

Several different subsets of DC have been characterized in mice and in humans, originating ontogenetically from myeloid or lymphoid precursor cells, respectively. Distinct immunomodulatory functions such as T helper 1 (Th1) versus T helper 2 (Th2) cell stimulation have been assigned to specific DC subsets. Increasing evidence is presented that functional diversity of discrete DC subsets cannot be exclusively explained by different lineage origin, but also depends on the activation signals they receive and on their maturation stage, culture conditions, and local cytokine environment (7). DC infiltration into tumors has been associated with prolonged survival and reduced metastasizing capacity in cancer patients (8,9).

Dendritic Cell Immunotherapy

Since large-scale isolation and expansion of DC in culture have become feasible, DC-based therapies have been successfully employed in several clinical trials for cancer, such as melanoma (10), renal cell carcinoma (11), and prostate cancer (12). Generally DC have been generated in culture either from peripheral blood non-proliferating $CD14^+$ monocytic precursors or proliferating $CD34^+$ progenitor cells by addition of appropriate cytokines such as IL-4, GM-CSF, TNF-α, c-kit ligand and Flt-3 ligand. Depending on the combination of added cytokines, either immature (e.g., $CD14^+$ monocytes incubated with GM-CSF/IL-4) or mature (e.g., $CD34^+$ stem cells cultivated with GM-CSF/TNF-α) DC are produced. DC yields of 1×10^6 cells per 10 ml blood and $\geq 5 \times 10^8$ cells per single leukapheresis have been achieved (13). Recently, a 20-fold expansion of DC in cancer patients *in vivo* by Flt-3 ligand treatment has been reported (14). Doses of up to 1×10^9 antigen-pulsed DC have been administered in cancer patients without occurrence of major side effects. Correlations between duration of immune response and numbers of administered DC have been seen in human and animal studies (15). It has remained a matter of debate whether DC derived from $CD34^+$ precursor cells or monocyte-derived DC should be preferentially used in clinical vaccination protocols. Both cell populations have been shown in culture to effectively induce antigen-specific T cell responses. Differences in their T cell priming capacity seemed to be due mainly to varying culture conditions (16). While choice of the optimal precursor cells for generation of clinically administered DC vaccines needs to be further examined, evidence was presented that cultured mature DC are superior to immature DC in eliciting both protective immunity and CTL responses against established tumors *in vivo* (17,18). Enhanced antigen presenting and chemotactic capacity of mature DC as well as secretion of IL-12 known to promote Th1 helper cell activation may account for this.

DC used in vaccination studies have been loaded in culture with specific tumor-associated peptides, tumor RNA and cDNA, tumor cell lysate or apoptotic tumor cells. Furthermore, recently allogeneic DC fused to autologous tumor cells have been used successfully as vaccines in patients with renal cell carcinoma (11). Using immunogens consisting of a panel of antigens instead of specific peptides not only reduces the likelihood of generating antigen loss variants in tumors, but also expands the scale of potentially available MHC-restricted epitopes, thus making these vaccines amenable to a broader range of patients. Additionally, these antigens most likely also provide MHC class II-restricted epitopes needed for induction of $CD4^+$ T helper cells. $CD4^+$ T cells are known to represent important regulators of sustained antitumor immunity (19).

Efficient T cell priming *in vivo* requires migration of DC to the T cell areas of lymphoid organs. The route of vaccine administration therefore seems to be of critical importance. Morse et al. (20) demonstrated that cultured and antigen-pulsed DC injected intravenously (i.v.) in cancer patients accumulate in the liver, spleen and bone-marrow, but cannot be detected in regional lymph nodes or tumor tissue. After intradermal (i.d.) injection, a small fraction of labeled DC where shown to migrate to draining lymph nodes, while DC administered subcutaneously (s.c.) were never detected in regional lymphatics. It cannot be ruled out that the latter was due to lack of sensitivity of the detection method, as DC given s.c. to mice could be detected in draining lymph nodes (21). In cancer patients DC immunotherapy has been administered either via a single route or as a combination of i.v., s.c., i.d. and intranodal (i.n.) administrations. Tumor antigen-specific T cell responses could be elicited in all treated patients. Regarding the trials employing single route administration, complete tumor responses in some patients have been reported for i.v., s.c. and i.n. DC vaccination (22). Although the optimal schedule for DC immunotherapy is still under investigation, a consensus was reached that DC vaccines should be administered repeatedly, as booster immunizations have been shown to strongly increase antigen-specific T cell responses (23).

Genetic engineering of DC in order to enhance their immunostimulatory capacity is now gaining increasing attention. Transgenes such as immunostimulatory cytokines (e.g., IL-12, GM-CSF), costimulatory molecules (e.g., B7-1, ICAM-1) and tumor-associated antigens (e.g., MART-1, MAGE-3)

have been delivered by viral and non-viral vectors into DC and were shown to substantially improve antigen-specific CTL responses (24,25). The reported efficiency of gene transfer varied considerably (10–95%), depending on the transfer system and the DC maturation stage and lineage origin (26,27).

DC Immunotherapy for Glioma

Glioma patients show severely impaired cellular immunity. Immunosuppressive factors secreted by the tumor, such as IL-10, TGF-β2 and prostaglandin E_2 are considered to be the main cause for this defect (28). Immunotherapeutic approaches may only be beneficial if the compromised immune status in these patients is overcome. Cellular immunotherapies such as adoptive transfer of lymphokine-activated killer cells (LAK), tumor-infiltrating lymphocytes (TIL), and tumor-sensitized CTL, as well as vaccination with irradiated tumor cells have been performed with limited success in glioma patients (29). Tumor-antigen pulsed DC used as vaccines *in vivo* offer the unique feature of presenting tumor-specific peptides in the context of an efficient antigen processing and presenting machinery and a multitude of costimulatory molecules, thus stimulating immune effector cells in a very potent way.

Specific recognition of tumor cells by infiltrating CTL is mediated by MHC-restricted presentation of antigens by glioma cells. In glioma, only few tumor-associated antigens representing targets for CTL have been described, e.g., MAGE-1, MAGE-3 and SART3 (30,31). Therefore, DC loaded with a panel of tumor-associated antigens such as tumor cell lysate, RNA, cDNA, or a mixture of peptides eluted from tumor cell membranes seem more promising treatment options than DC pulsed with defined tumor peptides. Accordingly, the animal studies and the three clinical studies reported to date used DC pulsed with a broad spectrum of glioma antigens.

Animal Studies of DC-based Immunotherapy for Glioma

The first study utilizing DC as vaccine for glioma was reported by Siesjö et al. (32). Rats harboring intracranial N32 gliomas were immunized with syngeneic spleen-derived DC mixed with irradiated N32 glioma cells or a mutagen-induced immunogenic tum^- N32 variant, respectively. After four vaccinations given every two weeks s.c. significantly prolonged mean survival time and some cures of treated rats could be demonstrated: while all control animals died within 40 days, 7 of 26 (27%; N32 vaccine) and 6 of 26 (23%; tum^- N32 vaccine) immunized rats were alive on day 200 after tumor challenge. Simultaneous i.v. injection of pulsed DC diminished this effect. Immunization with DC mixed with unrelated syngeneic brain tumor cells did not have any antitumor efficacy. Interestingly, all long-term survivors were females while prolonged survival was recorded only occasionally in male rats.

Effective treatment of intracerebral glioma with DC was also shown in the experimental rat 9L gliosarcoma model (33). Starting one week after intracranial tumor implantation, the animals received three weekly s.c. injections of bone-marrow generated DC pulsed ex vivo with acid-eluted antigens from 9L glioma cells. Survival was significantly increased compared to control animals receiving DC pulsed with normal rat astrocyte peptides. While 7 of 12 (58%) animals immunized with tumor antigen-pulsed DC were alive at day 31, none of the control animals receiving either medium or unpulsed or astrocyte peptide-pulsed DC survived. Three of 12 (25%) 9L antigen-pulsed DC-treated rats survived more than 60 days. Histological examination of their brains proved complete eradication of their tumors. No signs of inflammation or demyelination were seen outside the immediate peritumoral regions. Tumors in rats treated with tumor antigen-loaded DC showed moderate to heavy T cell infiltration, predominantly of $CD8^+$ T cells, while tumors of control animals displayed no T cell infiltrates.

A spontaneously arisen astrocytoma mouse model resembling phenotypically and morphologically human glioma was employed by Heimberger et al. (34). DC prepared from murine bone marrow were pulsed with homogenate from the 560 astrocytoma cell line transfected with the murine homologue of the mutated epidermal growth factor receptor (EGFRvIII). EGFRvIII is oncogenic and overexpressed in the majority of human glioblastomas. Mice were vaccinated i.p. with pulsed DC once a week for 4 consecutive weeks. One week later mice were challenged with EGFRvIII-transfected 560 cells injected intracerebrally (i.c.). While control animals treated with saline or unpulsed DC had a median survival of 26 and 25 days, respectively, 5 of 10 (50%) mice immunized with pulsed DC were long-term survivors with a significantly longer median survival ($>$65 days; $p = 0.016$). The five long-term survivors were rechallenged with glioma cells 50 days after initial challenge. All of these mice survived the rechallenge for more than 50 days. Splenocytes from immunized mice were capable of lysing the parental 560 and the EGFRvIII transfected 560 glioma cells in vitro but not HLA-matched fibroblasts transfected with EGFRvIII, demonstrating that EGFRvIII was not an immunodominant antigen in this system. Histologically, no intracerebral inflammation outside the tumor area was recorded. Although no data are presented, the authors report that treatment of established tumors with DC loaded with tumor homogenate did not increase median survival.

Yamanaka et al. (35) tested several strategies for antigen delivery into DC in the mouse 203 glioma model. DC were pulsed with either tumor RNA, tumor lysate or Semliki Forest virus (SFV)-mediated 203 glioma cDNA and injected into glioma bearing mice. SFV is a self-replicating RNA virus capable of infecting both dividing and non-dividing cells and

primarily used for transient high-level expression of proteins. Pulsed DC were administered i.p. 3 times spaced one week apart. Animals immunized with DC pulsed with saline, 203 glioma lysate, 203 glioma or B16 melanoma RNA, SFV-LacZ (β-galactosidase) or SFV-mediated B16 melanoma cDNA had median survival times between 20 to 35 days. Mice treated with DC pulsed with SFV-mediated 203 glioma cDNA showed increased median survival of 90 days ($p < 0.05$). Seven of 14 (50%) animals were still alive at day 90. Histologically, the brains of long-term survivors were tumor free. No chronic inflammation was detected in the brains of treated animals. Tumors of mice that had been treated with SFV-mediated 203 glioma cDNA showed increased infiltration of $CD8^+$ T cells. The induction of a significantly enhanced CTL response by DC loaded with SFV-mediated 203 glioma cDNA compared to DC pulsed with either glioma cDNA, glioma RNA or glioma lysate, which also elicited a CTL response, was also demonstrated in culture ($p < 0.01$). In the tumor challenge experiment 203 glioma cells were administered i.c. after 3 weekly intraperitoneal injections with DC. All mice treated with the above mentioned controls died, median survival being 35 to 40 days. Median survival of mice that were immunized with DC pulsed with SFV-mediated 203 glioma cDNA was greater than 90 days ($p < 0.05$), 6 of 12 mice (50%) were alive at day 90. Mice receiving this vaccine and challenged with B16 melanoma cells could not reject the tumor.

Immunization of mice with bone marrow-derived DC fused to glioma cells was recently examined by Akasaki et al. (36). DC were incubated for 48 hours with irradiated (50 Gy) SR-B10.A mouse glioma cells at a ratio of 1:3 followed by polyethylene glycol treatment to induce fusion. Tumor cells were dyed red with PKH26 and DC were stained for CD80. Forty percent double positive cells were detected by FACS analysis which was nearly identical to the percentage of CD80 positive cells in the mixed culture and interpreted by the authors as a proof for the presence of fused cells (FC). However, regarding the high percentage of fused (chimeric) DC, the protocol, and the detection method employed it can not be ruled out that double stained cells represent DC having engulfed apoptotic tumor cells rather than fused cells (37,38). In the tumor challenge model 1×10^5 fused cells (FC) were injected s.c. into syngeneic mice on day 0 and day 7. On day 14, parental glioma cells were injected either into the contralateral flank or i.c. Control mice treated with irradiated tumor cells died because of the growing tumor within 6 weeks. Mice treated with FC and challenged with tumor cells in the flank survived without apparent tumor growth. Of 20 mice immunized with FC and challenged i.c., 10 (50%) were long-term survivors ($p < 0.01$). Treatment with DC alone or with DC fused to irrelevant glioma cells did not enhance survival. In the tumor treatment model SR-B10.A glioma cells were injected i.c. on day 0 and FC were given s.c. on days 5 and 12. Although FC-treated mice showed increased survival, this was not statistically significant ($p > 0.05$). However, combined treatment of glioma-bearing mice with recombinant mouse IL-12 and FC prolonged survival significantly ($p = 0.01$): five of 10 mice (50%) survived for more than 70 days. IL-12 or DC treatment alone did not increase survival significantly. IL-12 is known to promote Th1 cell development and enhance $CD8^+$ T cell mediated cytotoxicity. Induction of CTL-mediated glioma-specific cytotoxicity in mice treated with FC was also demonstrated in cell culture. Simultaneous *in vivo* administration of IL-12 significantly enhanced cytotoxicity ($p = 0.002$). Mice depleted of $CD8^+$ T cells by treatment with anti-CD8 antibodies (>95% depletion) prior to tumor inoculation and FC treatment were not protected anymore from tumor development by the immunotherapy. Histological analysis in FC treated animals demonstrated strong infiltration of brain tumor tissue by $CD4^+$ and $CD8^+$ T cells.

Ni et al. (39) investigated dendritic cell-mediated immunotherapy by pulsing cloned DC2.4 DC originating from C57BL/6 mice with GL261 glioma cell extracts and injecting them i.p. into C57BL/6 mice with intracerebral syngeneic GL261 gliomas. The DC2.4 dendritic cell line had been established by transfecting GM-CSF-transduced bone marrow cells with *myc* and *raf* oncogenes (40). DC2.4 cells morphologically resemble DC, display normal phagocytic function, and express high levels of MHC class I and class II molecules and the costimulatory molecules CD80, CD86 and ICAM-1. Mice challenged with 4×10^4 GL261 cells i.c. were vaccinated on days 0, 3, 7, 10, and 14 with 2×10^6 DC2.4 cells pulsed with GL261 lysate. Survival of these animals after 100 days was 60% (6/10) and significantly augmented compared to controls immunized with either saline (30%, 3/12) or non-pulsed DC2.4 cells (25%, 2/8), $p < 0.05$. Cured animals showed an increased delayed-type hypersensitivity (DTH) response as measured by foot pad swelling in response to local injection of irradiated GL261 cells. The survival rate was inversely correlated to the initial tumor load: when mice received 2×10^5 glioma cells i.c. only 43% of DC-immunized animals were alive at day 100. Animals who survived for more than 120 days were rechallenged with GL261 cells i.c. Naïve controls died by day 40, while rechallenged animals survived past day 120 ($p < 0.05$).

Human Clinical Trials of DC-based Immunotherapy for Glioma

The promising results of DC vaccinations in animal glioma models were followed by first reports on DC immunotherapy in glioma patients. Liau et al. (41) published a case report on a patient with recurrent brain stem glioma who was immunized with autologous DC pulsed with tumor peptides. The tumor

had recurred 11 months after initial diagnosis. Peptides were isolated from an allogeneic MHC class I-matched primary glioblastoma cell line by acid treatment and loaded onto autologous DC which had been generated from peripheral blood leukocytes and cultured with GM-CSF and IL-4 for 7 days. After subtotal resection of the tumor the patient received three biweekly peptide-pulsed DC injections i.d. into the left axilla. No serious adverse effects associated with the immunotherapy were observed. Following treatment, a strong proliferation of the patient's T-cells in response to tumor peptide was seen. Brain tumor specimens obtained after the second immunization showed increased $CD3^+$ T cell infiltration into the tumor and no signs of experimental allergic encephalomyelitis (EAE). The tumor progressed 2 months after DC vaccination and the patient died 9 months after detection of the recurrent tumor.

The first phase I trial using DC-based vaccines for glioma patients has been reported recently by Yu et al. (42). Five women and 4 men were enrolled in this study; their age ranged from 28 to 77 years (mean age 49 years). Two patients had anaplastic astrocytoma, seven GBM, all were newly diagnosed. Prior to DC treatment patients had received surgical debulking of their tumors followed by standard dose radiation therapy (60 Gy). Forty-two control patients with newly diagnosed GBM underwent tumor resection and radiation therapy of 60 Gy with or without chemotherapy. At the time of DC vaccination all patients were off steroids. DC were prepared from the patients' peripheral blood and expanded in culture for seven days with IL-4 and GM-CSF. In all patients, more than 70% of the cells expressed the DC phenotype $HLA\text{-}DR^+$, $CD80^+$ and $CD14^-$. Their functionality was proved in allogeneic proliferation assays. Peptides from the surface of cultured autologous tumor cells were acid-eluted and incubated with autologous DC overnight prior to vaccination. 1×10^6 peptide-pulsed DC were injected i.d. in the deltoid region three times biweekly. The treatment was safe, no evidence of significant side effects or autoimmune toxicity occurred. DC vaccination proved to be associated with increased survival: median survival time for the study group and the control group were 455 days and 257 days, respectively ($p = 0.04$). Seven of the immunized patients were evaluated for development of systemic antitumor cytotoxicity: four of them showed in vitro an enhanced CTL response after DC vaccination. Four patients with progressive disease underwent tumor resection after the last immunization. Of these, two patients showed infiltration of CTL and $CD45RO^+$ memory cells into the tumor which was not seen in tumor specimens prior to vaccination. Increased $CD4^+$ T cells infiltrates were also reported, but were significantly less than $CD8^+$ T cell infiltrates. The tumor specimens of the other two reoperated patients did not show increased T cell infiltration, both patients died from progressive tumor disease. Tumor specimens from 4 non-vaccinated glioma patients undergoing reoperation because of recurrent disease also did not display increased T cell infiltration.

These authors (43) recently presented a second phase I trial supporting their initial findings. Seven patients with recurrent glioma and three patients with recurrent anaplastic astrocytoma were vaccinated i.d. three times biweekly with autologous DC pulsed with lysates of autologous tumor. Again, this therapy proved to be safe, no signs of an unwanted autoimmune response were seen. One vaccinated patient, who was tested for a systemic immune response demonstrated a strong antigen-specific CTL response as well as a strong T cell infiltration intratumorally. Median survival for the vaccinated group was 392 days and for the control group ($n = 51$) 153 days ($p = 0.003$).

Recently, Okada et al. (44) announced a DC immunotherapy trial for glioma patients. The vaccine to be administered i.d. consists of autologous irradiated tumor and dendritic cells as well as IL-4 transduced autologous fibroblasts. IL-4 is thought to enhance tumor antigen presentation and expression of costimulatory molecules on DC, DC influx, as well as to stimulate a long-lasting anti-tumor T cell response.

Discussion

The six animal studies of DC therapy for glioma can hardly be compared to each other due to the great differences in study design. Some of these differences are in the choice of animal (rat vs. mouse) and tumor model, the source (spleen vs. bone marrow and cloned cell line) and quality of DC (cytokines used for culturing of DC), the type of antigen (peptides, homogenate, RNA, whole cells), the administration route (s.c., i.p., i.v.), and in the applied DC dose and time schedule of vaccination.

Despite these differences important conclusions may be drawn from these animal experiments and from the clinical studies:

1. DC therapy of glioma is safe. No side effects and signs of systemic autoimmunity or chronic inflammation at the tumor site were reported (32–36,39,41–43).
2. DC immunotherapeutics can induce tumor rejection *in vivo* and may result in significantly prolonged survival in glioma-bearing animals and patients with glioma (32,33,35,36,39,42,43). In animal models even cures have been reported. Rates of long-term survivors among the rodents that received tumor-antigen pulsed DC ranged from 23% to 60% in these studies. However, the interpretation of the above data should be exercised with caution as the follow-up times in the survival studies are varying and the initial intracerebral tumor burden is different in the different studies. Although not showing data, only

Heimberger et al. (34) reported that their DC vaccination protocol did not increase survival of animals with i.c. established tumors. Due to the differences in study design it remains difficult to explain this failure of therapy. One major difference that emerges is that this group used male animals only. Four studies performed DC therapy only in female animals (33,35,36,39). Siesjö et al. (32) employed female and male rats, but increased survival of DC-vaccinated animals could only be demonstrated for female rats: while 6 of 13 (46%) female rats were long-term survivors, none of 13 male rats survived. Both humoral and cellular immunity are known to be stronger in females (e.g., higher rate of autoimmune diseases in females), most likely due to differences in hormonal regulation compared to men (45).

3. DC vaccination leads to protective and long-lasting immunity. All three groups (34–36) that performed tumor challenge experiments in mice after DC immunization reported long-term survival rates of 50%. Rechallenge experiments in long-term survivors showed that none of these animals died after a second injection of tumor cells (34,39).
4. DC therapy induces glioma-specific immune responses *in vivo*: astrocyte-pulsed DC (33) or DC pulsed with irrelevant tumors (32,35,36) did not prolong survival. *In vivo* depletion of $CD8^+$ T cells prior to DC vaccination abolished the inhibiting effect of glioma-antigen loaded DC on tumor growth (36). Generation of specific CTL-mediated cytotoxicity in immunized animals and patients was also demonstrated in cell culture (34,36,42, 43). DC pulsed with glioma-antigens induced a memory immune response in vaccinated animals as demonstrated by foot pad swelling after local injection of irradiated glioma cells (39). Tumors of animals and patients immunized with glioma antigen-pulsed DC demonstrated increased infiltration of T cells, predominantly $CD8^+$ and, to a lesser extent, also of $CD4^+$ T cells (33,35,36, 41–43). $CD45RO^+$ memory T-cells were also detected in these tumor specimens (42).
5. Success of DC therapy for glioma depends on initial tumor load. Ni et al. (39) recorded 60% long-term survivors when animals were injected with 4×10^4 glioma cells i.c. compared to 43% survivors when mice received 2×10^5 tumor cells i.c. before DC immunization.

Regarding female animals only, four of the groups studying DC therapy for established glioma reported long-term survivor rates between 43% and 60% when initiating DC therapy between day 0 and day 5 after tumor implantation (32,35,36,39). Liau et al. (33) found only 25% long-term survivors in their glioma model when starting DC therapy at day 7 after having administered a relatively high number of tumor cells (1×10^5 i.c.). Referring to the clinical use of DC immunogens for glioma patients, these results imply that DC therapy may have a stronger tumor controlling effect when administered in an adjuvant setting after gross tumor resection as performed in the 3 clinical studies (41–43).

DC immunization studies in glioma also confirmed aspects of DC therapy known from other DC cancer vaccination trials:

Source of DC

Five of the groups performing animal experiments used DC prepared from marrow precursor cells (33–36,39), while Siesjö et al. (32) used rat spleen-generated DC cultured without additional cytokines, indicating that DC from different origins can stimulate antitumor immune responses. As routinely done in other DC cancer vaccination trials, the three clinical studies of DC therapy for glioma (41–43) used DC derived from myeloid precursors. Two studies utilized DC-precursors prepared directly from peripheral blood and cultured in the presence of GM-CSF and IL-4, which enriches for immature monocyte-derived DC (41,42), while the third study (43) utilized DC derived from leukapheresis products, not mentioning in detail whether the patients had received cytokines (e.g., G-CSF) prior to leukapheresis and how the DC were cultured.

DC Maturation Stage

As far as can be deduced from the reported culture conditions for DC and the presented FACS data, the vaccines in all animal and human studies contained a variable proportion of terminally differentiated DC. Mature DC have been shown to be superior to their immature counterparts in eliciting antigen-specific T cell responses (18,23). Human DC prepared from monocytic precursors and cultured in GM-CSF and IL-4 as employed in the clinical trials (41,42) have been shown to give rise to a rather immature phenotype. It remains unknown whether antigen-loaded immature DC can mature *in vivo*. Remarkably, Heimberger et al. (34) who could only demonstrate success of DC therapy in the tumor challenge but not in the treatment model, used rat bone-marrow-generated DC cultured in GM-CSF without IL-4. Talmor et al. (46) showed that culturing rat marrow cells in GM-CSF without IL-4 primarily produced immature DC, while GM-CSF plus IL-4 gave rise to stimulatory, mature DC. Akasaki et al. (36) saw significantly increased median survival rates and cures in DC-vaccinated animals only if IL-12 was administered simultaneously. Forty percent of the DC coincubated with glioma cells before vaccination were reported to express CD80, a molecule which is strongly upregulated in mature DC. IL-12 has been shown to induce DC maturation and is the key cytokine secreted by mature DC. As uptake of apoptotic

cells is not a sufficient stimulus for DC maturation (47), it may be speculated that IL-12 is needed to compensate for the missing final maturation of the administered DC.

Route and Schedule of Administration

All studies used up to 4 booster vaccinations to augment tumor-specific immunity. Akasaki et al. (36) showed that tumor-specific CTL-responses are significantly increased if glioma-bearing animals receive two DC injections instead of one ($p < 0.001$). The reported doses administered to the animals (1×10^5 to 2×10^6 DC/injection) and the patients (1 or 5×10^6 DC/injection) are comparable to doses used successfully in other DC studies. Prolonged survival rates and even cures of tumor-bearing animals were achieved by injecting DC either s.c., i.p. (animal studies) or i.d. (human trials), implying that all three routes enable DC to get in close contact with effector T cells, e.g., by migrating to draining lymph nodes (21). Siesjö et al. (32) reported a negative effect on outcome when simultaneously administering 1×10^6 DC s.c. and i.v. (no long term-survivors) compared to s.c. injections of 1×10^6 DC (30% long term survivors). There is evidence that i.v. given DC predominantly accumulate in the spleen and are less effective in inducing CTL responses than DC administered s.c., i.p., or intralymphatically (21,48,49). On the other hand, as control injections of 2×10^6 DC given either s.c. or i.v. were not performed, it can not be ruled out that the negative effect of the higher DC dose on survival is simply due to over-vaccination leading to activation-induced T cell death.

Choice of Antigen

A multitude of antigen-loading strategies may be effective in inducing tumor-specific CTL responses. Sources of antigen used in the glioma experiments comprise intact tumor cells (32,36), acid-eluted peptides from tumor cell membranes (33,41,42), tumor cell homogenate (34,39,43) and tumor-derived mRNA (35). All methods of loading DC with antigen were capable of generating antitumor immunity. Yamanaka et al. (35) showed that in their model glioma RNA and glioma lysate were equal in inducing CTL responses in DC vaccinated mice, but that SFV-mediated glioma cDNA had a stronger stimulatory capacity and resulted in cures of glioma-bearing animals. Other studies demonstrated immunological equipotency also for DC pulsed with either tumor lysates, apoptotic or live cells, respectively (50).

Synthetic MHC class I peptides are rapidly degraded by human DC (51). This most likely applies as well to natural peptides eluted from tumor cell membranes and loaded onto DC (33,41,42). Liau et al. (33) found a rather low rate of 25% long-term survivors among animals vaccinated with peptide-pulsed DC. A patient immunized with DC pulsed with allogeneic MHC class I-matched glioblastoma peptides also showed a limited immunological response (41). Additionally, allogeneic tumor cell lines might present different immunodominant epitopes on their MHC class I molecules compared to the autologous tumor. MHC class I-restricted peptide mixtures do not contain $CD4^+$ T helper cell epitopes, which might also account for some of the failures seen with this type of antigen. Recombinant proteins or native full-length proteins derived from tumor cells may substantially enhance antitumor immunity by activating $CD4^+$ T cell help (33–36,39,43).

Preparation of tumor lysate, tumor mRNA, and acid-eluted membrane peptides requires sufficient amounts of tumor tissue. This is often limiting in a clinical setting. As amplification of mRNA by PCR can be easily performed, this method may be preferred if only small quantities of tumor tissue are available. Using recombinant antigens or allogeneic glioma cell lines might even circumvent the need for autologous tumor tissue.

Pools of antigens derived from tumor cells may also represent antigens expressed by normal cells and thus, at least in theory, may induce autoimmune toxicity. Although EAE could be elicited by administering human glioma to nonhuman primates and guinea-pigs (52), none of the DC studies in glioma recorded the occurrence of a severe autoimmune response. This corresponds to findings in many other animal and clinical trials for different types of cancer (5). Ludewig et al. (53) found that activation of self-reactive CTL by DC is limited by the rapid turnover and inefficient presentation of MHC class I associated peptides after uptake of exogenous cellular self-antigens. However, DC-based vaccinations with melanoma lysate induce widespread vitiligo in up to 43% of melanoma patients (54), implying that shared tumor antigens might indeed induce autoimmune responses against normal tissues and that caution is advisable when chosing the antigen.

Adjuvants/Cytokines

Akasaki et al. (36) showed that simultaneous use of IL-12 is an efficacious way of increasing CTL responses in glioma bearing mice. The T cell stimulatory effect of IL-12 on tumor-specific T cells has also been demonstrated in DC-based therapy for other tumors (55). Other cytokines, such as GM-CSF, IL-2, and IL-7, and helper antigens such as keyhole limpet hemocyanin (KLH) are known to enhance T cell responses in DC-based human and animal trials, and may therefore also be tested for efficacy in glioma. As systemic administration of cytokines is often accompanied by severe side effects, genetic engineering of DC to express stimulatory cytokines locally is increasingly used (24).

Gene-modified DC

Virally transduced DC loaded with the whole repertoire of tumor antigens were shown to be particularly potent in inducing a specific immune response

against glioma (35). Recombinant expression of tumor antigens enables continuous cross-presentation of exogenous tumor antigens on MHC class I molecules as they gain access to the intracellular antigen processing machinery. Gene transfer into DC by viral vectors is very efficient, although, in humans, the humoral immune response to adeno- and vaccinia viruses (due to previous viral infections or vaccination) may abolish the effect of booster vaccinations. Humoral immune responses against alphaviral vectors such as SFV have not yet been reported. SFV induces apoptosis in infected cells, which was also shown for glioma cells (35). Uptake of apoptotic cells by DC followed by processing and cross presentation of the exogenous antigens may account for the strong immunogenicity demonstrated with this vector system (56).

Conclusion

Dendritic cell therapy as an adjuvant treatment for patients with malignant glioma seems to be promising. As with DC vaccination trials for other malignancies, DC therapy of glioma proved to be biologically safe and without major side effects. Nevertheless, its efficacy remains to be more closely examined in randomized and controlled clinical trials. The development of advanced methods for genetic manipulation and transduction of DC prior to vaccination in humans may considerably increase the clinical benefits from this type of biological treatment.

References

1. Perrin G, Schnuriger V, Quiquerez AL, et al. (1999) Astrocytoma infiltrating lymphocytes include major T cell clonal expansions confined to the CD8 subset. *Int. Immunol.* **11:** 1337–1349.
2. Walker PR, Calzascia T, Schnuriger V, et al. (2000) The brain parenchyma is permissive for full antitumor CTL effector function, even in the absence of CD4 T cells. *J. Immunol.* **165:** 3128–3135.
3. Speiser DE, Miranda R, Zakarian A, et al. (1997) Self antigens expressed by solid tumors do not efficiently stimulate naive or activated T cells: implications for immunotherapy. *J. Exp. Med.* **186:** 645–653.
4. Steinman RM, Turley S, Mellman I, Inaba K. (2000) The induction of tolerance by dendritic cells that have captured apoptotic cells. *J. Exp. Med.* **191:** 411–416.
5. Schreurs MW, Eggert AA, Punt CJ, Figdor CG, Adema GJ. (2000) Dendritic cell-based vaccines: from mouse models to clinical cancer immunotherapy. *Crit. Rev. Oncog.* **11:** 1–17.
6. Hoffmann TK, Meidenbauer N, Dworacki G, Kanaya H, Whiteside TL. (2000) Generation of tumor-specific T-lymphocytes by cross-priming with human dendritic cells ingesting apoptotic tumor cells. *Cancer Res.* **60:** 3542–3549.
7. Grabbe S, Kämpgen E, Schuler G. (2000) Dendritic cells: multilineal and multi-functional, *Immunol. Today* **21:** 431–433.
8. Zeid NA, Muller HK. (1993) S100 positive dendritic cells in human lung tumors associated with cell differentiation and enhanced survival. *Pathology* **25:** 338–343.
9. Goldman, SA, Baker E, Weyant RJ, Clarke MR, Myers JN, Lotze MT. (1998) Peritumoral CD1a-positive dendritic cells are associated with improved survival in patients with tongue carcinoma. *Arch. Otolaryngol. Head Neck Surg.* **126:** 641–646.
10. Thurner B, Haendle I, Röder C, et al. (1999) Vaccination with MAGE-3A1 peptide-pulsed mature, monocyte-derived dendritic cells expands specific cytotoxic T cells and induces regression of some metastases in advanced stage IV melanoma. *J. Exp. Med.* **190:** 1669–1678.
11. Kugler A, Stuhler G, Walden P, et al. (2000) Regression of human metastatic renal cell carcinoma after vaccination with tumor cell-dendritic cell hybrids. *Nature Med* **6:** 332–336.
12. Lodge PA, Jones LA, Bader RA, Murphy GP, Salgaller ML (2000) Dendritic cell-based immunotherapy of prostate cancer: Immune monitoring of a phase II clinical trial. *Cancer Res.* **60:** 829–833.
13. Thurner B, Roder C, Dieckmann D, et al. (1999) Generation of large numbers of fully mature and stable dendritic cells from leukapheresis products for clinical application. *J. Immunol. Methods* **223:** 1–15.
14. Fong L, Hou Y, Rivas A, et al. (2001) Altered peptide ligand vaccination with Flt3 ligand expanded dendritic cells for tumor immunotherapy. *Proc. Natl. Acad. Sci. USA* **98:** 8809–8814.
15. Salgaller ML, Thurnher M, Bartsch G, Boynton AL, Murphy GP. (1999) Report from the International Union Against Cancer (UICC) tumor biology committee. *Cancer* **86:** 2674–2683.
16. Ferlazzo G, Klein J, Paliard X, Wei WZ, Galy A. (2000) Dendritic cells generated from $CD34^+$ progenitor cells with flt3 ligand, c-kit ligand, GM-CSF, IL-4, and TNF-alpha are functional antigen-presenting cells resembling mature monocyte-derived dendritic cells. *J. Immunother.* **23:** 48–58.
17. Labeur MS, Roters B, Pers B, et al. (1999) Generation of tumor immunity by bone marrow-derived dendritic cells correlates with dendritic cell maturation stage. *J. Immunol.* **162:** 168–175.
18. Jonuleit H, Giesecke-Tuettenberg A, Tuting T, et al. (2001) A comparison of two types of dendritic cell as adjuvants for the induction of melanoma-specific T-cell responses in humans following intranodal injection. *Int. J. Cancer* **93:** 243–251.
19. Wang RF. (2001) The role of MHC class II-restricted tumor antigens and CD4+T cells in antitumor immunitiy. *Trends Immunol.* **22:** 269–276.
20. Morse MA, Coleman RE, Akabani G, Niehaus N, Coleman D, Lyerly HK. (1999) Migration of human dendritic cells after injection in patients with metastatic malignancies. *Cancer Res.* **59:** 56–58.
21. Eggert AA, Schreurs MW, Boerman OC, et al. (1999) Biodistribution and vaccine efficiency of murine dendritic cells are dependent on the route of administration. *Cancer Res.* **59:** 3340–3345.
22. Morse M, Lyerly HK. (2000) Clinical applications of dendritic cell vaccines. *Curr. Opin. Mol. Ther.* **2:** 20–28.
23. Dhodapkar MV, Krasovsky J, Steinman RM, Bhardwaj N. (2000) Mature dendritic cells boost functionally superior $CD8^+$ T-cell in humans without foreign helper epitopes. *J. Clin. Invest.* **105:** R9–R14.
24. Kirk CJ, Mule JJ. (2000) Gene-modified dendritic cells for use in tumor vaccines. *Hum. Gene Ther.* **11:** 797–806.
25. Jenne L, Schuler G, Steinkasserer A. (2001) Viral vectors for dendritic cell-based immunotherapy. *Trends Immunol.* **22:** 102–107.
26. Van Tendeloo VF, Snoeck, HW, Lardon F, et al. (1998) Nonviral transfection of distinct types of human dendritic cells: high-efficiency gene transfer by electroporation into hematopoietic progenitor- but not monocyte-derived dendritic cells. *Gene Ther.* **5:** 700–707.
27. Ardeshna KM, Pizzey AR, Thomas NS, Orr S, Linch DC, Devereux S. (2000) Monocyte-derived dendritic cells do not proliferate and are not susceptible to retroviral transduction. *Br. J. Haematol.* **108:** 817–824.
28. Parney IF, Hao C, Petruk KC. (2000) Glioma immunology and immunotherapy. *Neurosurgery* **46:** 778–791.
29. Pollack IF, Okada H, Chambers WH. (2000) Exploitation of immune mechanisms in the treatment of central nervous system cancer. *Semin. Pediatr. Neurol.* **7:** 131–143.
30. Chi DD, Merchant RE, Rand R, et al. (1997) Molecular detection of tumor-associated antigens shared by human cutaneous melanomas and gliomas. *Am. J. Pathol.* **150:** 2143–2152.

31. Murayama K, Kobayashi T, Imaizumi T, et al. (2000) Expression of the SART3 tumor-rejection antigen in brain tumors and induction of cytotoxic T lymphocytes by its peptides. *J. Immunother.* **23:** 511–518.
32. Siesjö P, Visse E, Sjogren HO. (1996) Cure of established, intracerebral rat gliomas induced by therapeutic immunizations with tumor cells and purified APC or adjuvant IFN-γ treatment, *J. Immunother.* **19:** 334–345.
33. Liau LM, Black KL, Prins RM, et al. (1999) Treatment of intracranial gliomas with bone marrow-derived dendritic cells pulsed with tumor antigens. *J. Neurosurg.* **90:** 1115–1124.
34. Heimberger AB, Crotty LE, Archer GE, et al. (2000) Bone marrow-derived dendritic cells pulsed with tumor homogenate induce immunity against syngeneic intracerebral glioma. *J. Neuroimmunol.* **103:** 16–25.
35. Yamanaka R, Zullo SA, Tanaka R, Blaese M, Xanthopoulos K. (2001) Enhancement of antitumor immune response in glioma models in mice by genetically modified dendritic cells pulsed with Semiliki Forest virus-mediated complementary DNA. *J. Neurosurg.* **94:** 474–481.
36. Akasaki Y, Kikuchi T, Homma S, Abe T, Kofe D, Ohno T. (2001) Antitumor effect of immunizations with fusions of dendritic and glioma cells in a mouse brain tumor model. *J. Immunother.* **24:** 106–113.
37. Jenne L, Arrighi JF, Jonuleit H, Saurat JH, Hauser C. (2000) Dendritic cells containing apoptotic melanoma cells prime human $CD8^+$ T cells for efficient tumor cell lysis. *Cancer Res.* **60:** 4446–4452.
38. Shaif-Muthana M, McIntyre C, Sisley K, Rennie I, Murray A. (2000) Dead or alive: immunogenicity of human melanoma cells when presented by dendritic cells. *Cancer Res.* **60:** 6441–6447.
39. Ni H, Spellman SR, Jean WC, Hall WA, Low WC. (2001) Immunization with dendritic cells pulsed with tumor extract increases survival of mice bearing intracranial gliomas. *J. Neuro-Oncol.* **51:** 1–9.
40. Shen Z, Reznikoff G, Dranoff G, Rock KL. (1997) Cloned dendritic cells can present exogenous antigens on both MHC class I and class II molecules. *J. Immunol.* **158:** 2723–2730.
41. Liau LM, Black KL, Martin NA, et al. (2000) Treatment of a glioblastoma patient by vaccination with autologous dendritic cells pulsed with allogeneic major histocompatibility complex class I-matched tumor peptides. Case report. *Neurosurg. Focus* **9:** 1–5.
42. Yu JS, Wheeler CJ, Zeltzer PM, et al. (2001) Vaccination of malignant glioma patients with peptide-pulsed dendritic cells elicits systemic cytotoxicity and intracranial T-cell infiltration. *Cancer Res.* **61:** 842–847.
43. Yu JS, Wheeler CJ, Zeltzer PM, et al. (2001) Dendritic cell immunotherapy for patients with glioblastoma multiforme and anaplastic astrocytoma. *Proc. Am. Assoc. Cancer Res.* **42:** Abstract 1478.
44. Okada H, Pollack IF, Lieberman F, et al. (2001) Gene therapy of malignant gliomas: a pilot study of vaccination with irradiated autologous glioma and dendritic cells admixed with IL-4 transduced fibroblasts to elicit an immune response. *Hum. Gene Ther.* **12:** 575–595.
45. Gaillard RC, Spinedi E. (1998) Sex- and stress-steroids interactions and the immune system: evidence for a neuroendocrine-immunological sexual dimorphism. *Domest. Anim. Endocrinol.* **15:** 345–352.
46. Talmor M, Mirza A, Turley S, Mellman I, Hoffman LA, Steinman RM. (1998) Generation of large numbers of immature and mature dendritic cells from rat bone marrow cultures. *Eur. J. Immunol.* **28:** 811–817.
47. Sauter B, Albert ML, Francisco L, Larsson M, Somersan S, Bhardwaj N. (2000) Consequences of cell death: exposure to necrotic tumor cells, but not primary tissue cells or apoptotic cells, induces the maturation of immunostimulatory dendritic cells. *J. Exp. Med.* **191:** 423–433.
48. Serody JS, Collins EJ, Tisch RM, Kuhns JJ, Freilinger JA. (2000) T cell activity after dendritic cell vaccination is dependent on both type of antigen and the mode of delivery. *J. Immunol.* **164:** 4961–4967.
49. Fong L, Brockstedt D, Benike C, Wu L, Engleman EG. (2001) Dendritic cells injected via different routes induce immunity in cancer patients. *J. Immunol.* **166:** 4254–4259.
50. Lambert LA, Gibson GR, Maloney M, Barth RJ. (2001) Equipotent generation of protective antitumor immunity by various methods of dendritic cell loading with whole cell tumor antigens. *J. Immunother.* **24:** 232–236.
51. Amoscato AA, Prenovitz DA, Lotze ML. (1998) Rapid extracellular degradation of synthetic class I peptides by human dendritic cells. *J. Immunol.* **161:** 4023–4032.
52. Bigner DD, Pitts OM, Wikstrand CJ. (1981) Induction of lethal experimental allergic encephalomyelitis in nonhuman primates and guinea pigs with human glioblastoma tissue. *J. Neurosurg.* **55:** 32–42.
53. Ludewig B, McCoy K, Pericin M, et al. (2001) Rapid peptide turnover and inefficient presentation of exogenous antigen critically limit the activation of self-reactive CTL by dendritic cells. *J. Immunol.* **166:** 3678–3687.
54. Nestle FO, Banchereau J, Hart D. (2001) Dendritic cells: on the move from bench to bedside. *Nat. Med.* **7:** 761–765.
55. Fallarino F, Uyttenhove C, Boon T, Gajewski TF. (1999) Improved efficacy of dendritic cell vaccines and successful immunization with tumor antigen peptide-pulsed peripheral blood mononuclear cells by coadministration of recombinant murine Interleukin-12. *Int. J. Cancer* **80:** 324–333.
56. Ying H, Zaks TZ, Wang RF, et al. (1999) Cancer therapy using a self-replicating RNA vaccine. *Nat. Med.* **5:** 823–827.