T CELL IMMUNITY IN PATIENTS WITH MALIGNANT GLIOMA: RECENT PROGRESS IN DENDRITIC CELL-BASED IMMUNOTHERAPEUTIC APPROACHES

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1. ABSTRACT

Despite dramatic advances in surgical technique, imaging, and adjuvant radiotherapy or chemotherapy, the prognosis for patients with malignant glial tumors remains dismal. Based on the current knowledge regarding immune responses in the healthy central nervous system (CNS) and glioma-bearing hosts, we discuss dendritic cell (DC)-based immunotherapeutic approaches for malignant gliomas and the relevance of recent clinical trials and their outcomes. It is now recognized that the CNS is not an immunologically tolerated site, and clearance of arising glioma cells is a routine physiological function of the normal, non-compromised immune system. To escape from immune surveillance, however, clinically apparent gliomas develop complex mechanisms that suppress tumoricidal immune responses. Although the use of DCs for the treatment of glioma patients may be the most appropriate approach, an effective treatment paradigm for these tumors may eventually require the use of several types of treatment. Additionally, given the heterogeneity of this disease process and an immune-refractory tumor cell population, the series use of rational multiple modalities that target different tumor characteristics may be the most effective therapeutic strategy to treat malignant gliomas.
settings (3-6), therapeutic strategies that seek to stimulate immune recognition and clearance of glioma cells have the potential to effect powerful immune-mediated killing of all intracranial neoplastic foci. Many approaches seeking to bolster anti-tumor immunity using a variety of immunotherapies have been tested in rodent brain models. Although many of these strategies have proven highly effective in animals, their translation into human patients has not been as successful. Nevertheless, promising data are presented in the literature, and clinical trials of dendritic cell (DC)-based immunotherapy for patients with malignant brain tumors are currently underway. Based on the current knowledge regarding immune responses in the healthy central nervous system (CNS) and glioma-bearing hosts, we discuss DC-mediated treatment approaches for malignant gliomas and the relevance of recent clinical trials and their outcomes.

3. IMMUNE RESPONSES IN THE CNS

3.1. Immunological privilege of the CNS

The CNS had long been considered an immunologically tolerated site, due to a) the lack of lymphatic drainage, b) the nature of the blood-brain barrier (BBB), in which tight junction between cerebral vascular endothelial cells form a physical barrier to the passage of cells and antibodies, and c) the relative lack of MHC expressions in the healthy organ (7, 8). Despite such a peculiar microenvironment that hinders the operation of the immune system, it has become evident that a variety of leukocyte types, including T cells, B cells, and NK cells, continuously traffic though the CNS as part of normal immune surveillance (9). Most research on leukocyte entry into the CNS from their circulation pools has been directed toward understanding the mechanisms of T cell-mediated inflammation. The mechanisms of immune responses in multiple sclerosis, viral encephalitis, and a number of experimental systems, especially experimental autoimmune encephalomyelitis (EAE) underlie the rationale of the current understanding of CNS immunity (10-14). Based on research into these diseases, activated T cells are allowed to enter the parenchyma of the brain as the first step in the cascade toward CNS inflammation (Figure 1A). When the T cells recognize an antigen, the inflammatory pathway enters a second step (Figure 1B) (15), otherwise T cells leave the brain or undergo apoptosis upon contact with local APCs (Figure 1A) (16, 17). The phenomenon that T cells are inhibited by the anti-proliferative effect of gangliosides (18), TGF-β(19), and other signals (20, 21), may also be relevant to the mechanism of anti-inflammatory responses in the CNS. During the second step, T cells enwrapped in the CNS elaborate chemokines, such as MIP-1α and MCP-1 to recruit various leukocytes into the inflammatory site (Figure 1B) (22, 23). If the T cells succeed in recruiting effective leukocytes such as NK cells, macrophages, and Th1 cells in the third step, CNS inflammation may become clinically apparent (9). When the T cells are eliminated by the signals in the microenvironment before they recruit effective supports, the inflammation may be terminated (Figure 1C). Furthermore, if the recruited leukocytes exhibit tolerogenic responses, the inflammation may also be extinguished (Figure 1C) (14, 24). Thus, it is currently believed that the CNS does not bar the infiltration of peripheral circulating leukocytes but regulates the responses of infiltrating T cells in each of the multiple steps.

3.2. APCs for immune surveillance in the CNS

Primary brain tumors rarely generate extracranial metastasis. Nevertheless, T cell reactivity against tumor associated antigens (TAAs) has been demonstrated in the cervical lymph nodes of brain-tumor-bearing mice (25, 26). The reactivity of TAA-specific T cells is contingent upon effective antigen uptake by APCs. This requires either the presence of resident APC populations or trafficking of exogenous APCs into the brain. Microglia have been recognized as the native APCs and although their presence within malignant gliomas is well established, the antigen presenting function of microglia have been shown to be compromised, possibly due to down-regulation of cell surface MHC expression (27). In this context, it is likely that peripheral APCs that traffic into the CNS are responsible for effective antigen presentation to T cells. The perivascular monocytic cells are thought to arrive from a bone marrow source and accumulate signals from the microenvironment to become mature APCs (8, 9). Schneider et al. described the presence of numerous CD11c+ macrophages that strongly expressed HLA-DR and were morphologically distinct from microglia within multiple human glioma specimens (28). Fischer et al. demonstrated that functionally matured CD11c+ myeloid DCs appear and expand at perivascular and intraparenchymatic inflammatory sites concomitant to resident microglia proliferation (29). Furthermore, Yang et al. demonstrated the presence of OX-62 expressing DC populations within experimental rodent glioblastomas, strongly suggesting a role for endogenous tumor-infiltrating DCs as APCs in glioma (30). Based on these results, it is likely that peripheral circulating precursors of DCs enwrapped in the perivascular area have an ability to differentiate into mature DCs in the brain parenchyma. This differentiation may be caused by various microenvironmental signals. These exogenous DCs may participate in the maintenance of CNS immune response concomitant with resident APCs.

4. IMMUNITY IN PATIENTS WITH MALIGNANT GLIOMA

4.1. Suppressive effect of malignant gliomas on the immune system

It has recently been identified that glioma cells express various TAAs (31-38), which CTLs can recognize (Table 1). Given the already described ability of the immune system to generate antigen-specific T cell responses in the non-compromised CNS, TAA-expressing gliomas may be eliminated prior to becoming clinically apparent (Figure 2A). Despite this hypothesis, TAA expression was frequently observed on tumor tissues and primary cultured tumor cells established from surgical specimens of glioma patients (32, 38), indicating that these tumor cells have the potential to escape immune surveillance. Although it has been described that clinically apparent cancers are equipped to suppress host T cell immunity (39), it is more plausible that immunodeficient
Figure 1  Schema of immune responses in the CNS  (A) Activated leukocytes are allowed to enter the parenchyma of the brain as the first step in the cascade toward CNS inflammation. When the leukocytes do not recognize any antigen, they leave the brain or undergo apoptosis upon contact with local APCs. The phenomenon that leukocytes are inhibited by the anti-proliferative effect of gangliosides, TGF-β, and other signals may also be relevant to the mechanism of anti-inflammatory responses in the CNS.  (B) When the leukocytes recognize an antigen, the inflammatory pathway enters a second step. During the second step, leukocytes enwrapped in the CNS elaborate chemokines, such as MIP-1α and MCP-1 to recruit various leucocytes into the inflammatory site.  (C) When the leukocytes are eliminated by the signals in the microenvironment before they recruit effective supports, the inflammation may be terminated. Furthermore, if the recruited leukocytes exhibit tolerogenic responses, the inflammation may also be extinguished.
immunity in glioma patients with DC vaccine

Table 1. CTL-recognizing TAAas in glioma cells

<table>
<thead>
<tr>
<th>Antigens</th>
<th>HLA-restriction</th>
<th>Epitope Sequence</th>
<th>Detected Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAGE-1</td>
<td>A1</td>
<td>EADFTGHSV</td>
<td>U-87, U-118, U-373, U-138, IR-801, IR-802, IR-803, Primary glioma cells</td>
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<tr>
<td>AIM-2 (Spliced)</td>
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<td>Not shown</td>
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<tr>
<td>gp100</td>
<td>A2</td>
<td>TDFQVPSV</td>
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<td>A2</td>
<td>SVYDFWVVL</td>
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<td>A2</td>
<td>TIMAFQWVT</td>
<td>B2-17, U-251, KINGS-1, T98-G, KALS-1, KNS60, KNS81, ONS76, Normal brain tissue,</td>
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<td></td>
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<tr>
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<td>Glioma cell lines, Normal brain tissue, Primary glioma cells</td>
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</table>

individuals display a dramatic increase in cancer incidence. In this regard, it has been shown that gliomas have a higher incidence in immunodeficient states, such as HIV infection and post-transplant administration of immunosuppressive drugs (40-42). Tacconi et al reported that the frequency of gliomas in patients with AIDS-related focal mass lesions was approximately 6% (40). These findings strongly suggest the significance of T cell immunity for the immune system’s ability to eliminate arising glioma. Although it has already been shown that a variety of glioma-associated mediators induce the host cellular immune suppression (43, 44), these mechanisms are not fully understood. Glioma cells that successfully evade the initial immune killing are able to propagate into established tumors. As they proliferate, these tumor cells accumulate additional mutations, which may confer additional immuno- evade potentials involving the ability to: a) suppress endogenous APC function (45, 46); b) inactivate tumoricidal CTL responses (47, 48); and c) induce tolerogenic CD4+ T cell response (49). Unfortunately, when the glioma acquires one or more capabilities to suppress tumoral immune responses, tumor cells can survive and develop into a clinically apparent tumor (Figure 2B) (39).

4.2. Endogenous APC function in patients with malignant glioma

It has already been shown that both circulating and intratumorally infiltrating DCs are functionally impaired in tumor-bearing animals, and in cancer patients (50-53). In phase I clinical trials of DC-based immunotherapy for glioma patients, it has also been determined that defects in endogenous antigen presentation underlies the impaired cellular immunity seen in glioma patients (54-56). In this context, in vitro experiments on human gliomas revealed their ability to alter the function of peripheral blood mononuclear cell (PBMC)-derived DCs (46, 57). Specifically, DCs were cultured with glioma culture supernatants, and this insult resulted in reduced expression of MHC class II, CD80, and CD86 on the DCs. These DCs also impaired IL-12 production and instead increased IL-10 production (46). Although the identification of glioma-associated mediators for these suppressive effects was not determined in these experiments, these results indicate the possibility that endogenous APCs serve as an immune suppressive mediator between glioma-associated factors and T cell responses in glioma patients (Figure 2B).

4.3. Inactivation of tumoricidal CTL responses in the tumor microenvironment

It has long been believed that glioma-associated factors that inactivate tumoricidal CTL responses are a principal reason why gliomas escape immune surveillance. Expressions of Fas ligand (58), B7-H1 (59), and HLA-G (60), have been found to be immune suppressive factors on the cell surface of gliomas, where they probably contribute to inactivation of T cells through direct interaction. On the other hand, CTL inactivation is thought to be relevant to decreased expression of IL-2, IL-12, IFN-γ, and TNF-α, as well as increased expression of IL-4, IL-5, IL-6, IL-10, and TGF-β(47, 48, 61). In recent reports, it has been described that these alterations of cytokine profiles have been observed on tumor-exposed APCs in the tumor microenvironment as well as on tumor cells (46, 49). In the mechanism of CNS immune surveillance, TAA-recognition by the tumor-infiltrating CTLs is followed by the recruitment of tumoricidal supports. If those CTLs fail to recruit effective mediators such as IL-2, IL-12, IFN-γ, and TNF-α, they may be eliminated by various signals from the gliomas and/or glioma-exposed APCs, and then tumor cells can survive to be a clinically apparent glioma. Thus, TAA-specific CTL responses are regulated by not only factors produced by gliomas but also by endogenous APCs that may be altered functionally by glioma-associated mediators in the tumor microenvironment (Figure 2B).

4.4. Tolerogenic CD4+ T cell response in the glioma patients

Although the involvement of tolerogenic T cell responses may be of particular relevance in the setting of cancers (62), the mechanism of their induction in cancer patients remains unclear. Several tolerogenic T cell subsets have been identified within CD4+ cell populations (Table 2). Tr1 is characterized by secretion of IL-10 and TGF-β with negligible production of IL-4 (63, 64). Th3 is also characterized by secretion of IL-10 and TGF-β but differ from Tr1 in their dependence on IL-4 for functional differentiation (65, 66). In addition, a separate category of suppressor CD4+ T cells, identifiable by their co-expression of CD25, has also been described (67). The interrelationship of these regulatory subsets remains unclear, although they have long been known to induce peripheral T cell tolerance caused by the suppression of activated Th1 and Th2 cells (64), as well as by induction of...
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Figure 2. Schema of immune surveillance and escape mechanism on glioma. (A) The reactivity of TAA-specific T cells against glioma is contingent upon effective antigen uptake by APCs in the brain. This requires either the presence of resident APC (microglia) populations or trafficking of exogenous APCs (peripheral DCs) into the brain. These brain DCs may participate in the maintenance of CNS immune response by generating TAA-specific CD4+ and CD8+ T cell responses. (B) As glioma cells proliferate, they accumulate mutations, which may confer immuno-evasive potential. Gliomas create a complex microenvironment to escape from immune surveillance, in which they probably produce various factors to alter the host’s immune response against tumor cells, as well as to suppress tumoricidal CTL responses through direct effect.
apoptosis in circulating pools of activated CTLs (68). It is now recognized that apart from generating anti-tumor immune responses, DCs are also capable of initiating tolerogenic CD4+ T cell responses to inciting antigens (69, 70). In the absence of inflammatory signals, antigen-presenting DCs remain immature while entering the regional lymph nodes, and those immature DCs induce tolerance by deletion of antigen-specific T cell clones (71, 72). On the other hand, it has been shown that antigen-processing mature DCs can also induce antigen-specific tolerance, which is mediated by antigen-specific T regulatory subsets (68, 73). In this context, the influence of prostaglandin E2 (PGE2) on DCs has been linked to inhibited T cell responses (74). The overexpression of COX-2, the key enzyme governing synthesis of PGE2 from arachidonic acid (75), has also been well characterized in malignant gliomas (76, 77). These findings indicate that defective antigen presentation in the setting of glioma may be linked to the effect glioma-expressed mediators have on DCs, which could skew the immune response away from tumoricidal subsets to a tolerogenic regulatory phenotype. In this regard, we have recently reported a mechanism of Tr1 induction by mature DCs that phagocytose COX-2 overexpressing glioma (49). In this report, we observed that glioma-exposed mature DCs expressed high levels of IL-10, while their IL-12 production was significantly impaired. These DCs induced a Tr1 response. Selective COX-2 inhibition in COX-2 overexpressing glioma at the time of phagocytic uptake by DCs abrogated this regulatory response and instead elicited Th1 activity. The effect of COX-2 inhibition in COX-2 overexpressing glioma is reversible after administration of PGE2, indicating that glioma-associated PGE2 altered DC function to induce a Tr1 response. This tolerogenic Tr1 response probably exerts prominent regulatory effects against anti-tumor immune responses in the CNS, and may have a particular relevance to the observation that cellular immune function is frequently depressed at both the local intratumoral and systemic levels in glioma patients (Figure 2B) (78).

5. PRE-CLINICAL STUDIES OF DC-BASED IMMUNOTHERAPY FOR GLIOMAS

5.1. Experimental DC-based immunotherapy for brain tumors

Substantial evidence has accumulated that implies that CTLs are crucial to the generation of effective anti-tumor immune responses (79, 80). This recognition spurred the search for treatment modalities that could boost tumor-directed T cell responses. Although nonspecific cytokine therapy is capable of supporting and enhancing T cell activity (81), therapeutic strategies to generate TAA-specific immunity from naive T cells are more promising for researchers seeking to induce effective anti-tumor responses (82). Early vaccination strategies for glioma relied on subcutaneous inoculation of growth-arrested whole tumor cells. Although promising data has been obtained from tumor-cell vaccines (83, 84), the main criticism of these vaccines is that they rely on the inherent antigen presenting capacity of glioma cells, which is poor (85, 86).

There is now considerable evidence to support the contention that the use of professional APCs to initiate tumor-specific T cell responses is a more promising strategy for developing a glioma vaccine. In particular, therapeutic strategies involving DCs, characterized by the presence of large numbers of APC molecules such as MHC class I & II, CD80, and CD86, have received increasing attention (82, 87), and the ability of DCs to generate anti-tumor immune responses in the CNS has been documented (88-93). Most of these experiments have involved in vitro isolation of DCs, followed by loading of the DCs with TAAs and injection of the TAA-bearing DCs into syngeneic animals as a glioma vaccine. Bone-marrow-derived DCs pulsed with tumor lysate (88, 93), tumor cDNA (89, 92), apoptotic tumor cells (91), and DCs fused with tumor cells (90), have all been demonstrated to generate tumor-specific immune responses and anti-tumor activity in the brain. In particular, effector functions of CD8+ and CD4+ T cells have been shown to recognize and kill tumor cells. In experiments of DC-based immunotherapy, antigen-exposed DCs are expected to express multiple TAA-derived epitopes in combination with MHC Class I and II molecules on their cell surface. Consequently, this vaccine has the potential to induce immune responses against unidentified multiple TAAs. Moreover, DC-based immunotherapy may maintain protective immunological memory against subsequent tumor challenges (94). These encouraging results led to clinical trials that are currently underway.

5.2. Therapeutic strategies to induce tumor-specific Th1 response

Although traditionally CD8+ CTLs have been the focus of cancer immunotherapy, numerous studies have demonstrated that CD4+ T cells make a critical contribution to the development of effective anti-tumor immune responses (95, 96). CD4+ T cell activity helps create effective anti-tumor immunity, which is believed to be performed mainly by an antigen-specific Th1 subset. This subset occasionally interacts with resident APCs to activate them in the tumor microenvironment. This interaction may confer upon Th1 and resident APCs an ability to activate cellular immune responses (Figure 3A, B). Specifically, upon interaction between antigen-specific Th1 and DCs, which is mediated by the adhesion of TCR/CD4 complex to MHC class II, cellular immune responses are boosted by up-regulation of MHC and costimulatory molecule
Immunity in glioma patients with DC vaccine

**Figure 3. Interaction of CD4⁺ T cells with DCs**  
(A) Interaction between Th1 and DCs is mediated by the adhesion of the TCR/CD4 complex to MHC class II. Upon this interaction, cellular immune responses are adamantly boosted by up-regulation of MHC and costimulatory molecule expression, and additional adhesion such as CD40-CD40L and B7-CD28, cytokine signals such as IL-2 and IFN-γ from Th1, and IL-12 from DCs.  
(B) Th1 and resident APCs have the potential to stimulate each other to support TAA-specific CTL responses. The development of a successful anti-glioma vaccine may depend on the helper activity of the antigen-specific Th1 subset, which can interact with resident APCs to activate them in the tumor microenvironment.
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demonstrated robust infiltration of CD8+ and CD45RO+ T cells in patients with malignant tumors, however, frequently polarize away from the Th1 response toward Th2 responses or regulatory subsets (49, 100, 101). Therefore, therapeutic strategies to induce tumor-specific Th1 responses may have a particular relevance to the development of effective anti-tumor immunotherapy. In this regard, IL-12 and IL-18 may be ideal tools to enhance Th1 responses (90, 92). IL-12 is known as a heterodimeric cytokine that plays a central role in promoting Th1 responses and cellular immunity (102). IL-18 also stimulates Th1 responses in collaboration with IL-12 (103). Thus, IL-12 and its collaboration with IL-18 can enhance anti-tumor responses that are induced by DC-based immunotherapy. IL-23 has also recently been identified as an effective cytokine that supports cellular immunity by means of enhancing memory Th1 responses (104-107), indicating that it may be an ideal tool for DC-based immunotherapeutic strategies. As part of a strategy to improve DC function to generate effective Th1 responses, Liu et al. used RNA interference technology to modulate the function of PBMC-derived DCs (108). They demonstrated that transfection of DCs with IL-10-specific double stranded siRNA resulted in potent suppression of IL-10 production without inducing DC apoptosis or blocking DC maturation, and also increased IL-12 production after their maturation. It has also been shown that these DC alterations were accompanied by an augmented ability of DCs to boost Th1 responses. Given the already demonstrated ability of endogenous IL-10 to ultimately terminate cellular immune responses (109), DCs modified by IL-10 specific siRNA may be a promising device for cases that are resistant to DC-based immunotherapy.

6. CLINICAL TRAILS OF DC-BASED IMMUNOTHERAPY

6.1. Phase I/II clinical Trials of DC-based Immunotherapy

Yu et al. described the use of a DC vaccine in a phase I clinical trial involving patients with newly diagnosed high-grade glioma (54). DCs cultured from patients’ PBMC were pulsed ex vivo with autologous tumor cell surface peptide isolated by means of acid-elution. Nine patients (2 with anaplastic astrocytoma and 7 with GBM) received a series of three intradermal DC vaccinations following surgical resection and external beam radiotherapy. Anti-tumor cytototoxicity, as determined by exposing PBMC to autologous tumor targets as part of a JAM assay, was detected in 4 patients. Radiologic evidence of disease progression was detected in 4 patients, who then underwent re-operation subsequent to the third DC vaccination. In 2 patients, the harvested tissue demonstrated robust infiltration of CD8+ and CD45RO+ T cells, which was not apparent in the same patients’ tumor specimens resected prior to initiation of the vaccination protocol. Additionally, long-term survival in the study group was compared to data from age- and grade-matched controls with similar disease who underwent surgical resection with external beam radiotherapy. The median survival for the study group was 455 days vs 257 days for the control population, indicating that DC vaccination may confer some survival benefit. No destructive autoimmune response was observed in this study. These data indicated that DC-based immunotherapy for malignant glioma patients was safe and effective in stimulating anti-tumor immune responses as assessed by peripheral cytotoxicity assays and intratumoral T cell infiltration.

These encouraging results led to the expansion of this study into phase II trials, which were reported in 2004 (110). In the phase II trial, 14 patients (4 with anaplastic astrocytoma and 10 with GBM) were thrice vaccinated two weeks apart with autologous DCs pulsed with tumor lysate. One or more TAA-specific CTL clones against MAGE-1, pg100, and HER-2 were established in four of nine patients as part of a HLA-restricted tetramer staining assay. The median survival for the study group was 133 weeks vs 30 weeks for the control population, indicating that significant survival benefit was conferred by DC-based immunotherapy.

As part of a strategy to improve DC-mediated TAA presentation by means of enhancing tumor cell-DC interaction, Kikuchi et al used DC-glioma fusion cells in a phase I clinical trial (56). Eight patients (2 with anaplastic astrocytoma, 5 with GBM and 1 with anaplastic ologodendroglioma) were treated with a series of between 3 and 7 peripheral intradermal vaccinations with DC-autologous glioma fusion cells. Although only minor and temporary responses to therapy were detected in neuroimaging examinations of 2 patients who subsequently developed progressive disease, the capability of this vaccination to induce safely a tumor-specific immune response was demonstrated.

Based on the results of a mouse brain tumor model showing that systemic administration of recombinant IL-12 enhanced the anti-tumor effect of this vaccine (90), Kikuchi and colleagues subsequently reported clinical trials of vaccine therapy using DC-glioma fusion cells and recombinant human IL-12 (111). In this trial, 15 patients (7 with anaplastic astrocytoma, 6 with GBM and 2 with anaplastic oligoastrocytoma) who had progression of their tumor despite radiotherapy and/or chemotheraphy received vaccine therapy. The vaccination was scheduled to inject fusion cells intradermally close to a cervical lymph node on day 1, and then recombinant IL-12 (30ng/kg) was injected subcutaneously at the same site on days 3 and 7. This cycle was repeated every 2 weeks for 6 weeks, and a second 6 weeks course was repeated beginning 2 to 5 weeks after the last dose of IL-12 during course 1. As part of a 51Cr releasing cytolytic assay using peripheral blood lymphocytes and autologous glioma cells, cytolytic activity significantly increased after treatment in two patients, while that of other patients was almost nonexistent. In one patient, the activity after the treatment was lower than that before the treatment. Many larger tumor cells containing multiple nuclei and wide cytoplasm were observed in pathologic findings of recurrent tumor specimens, and
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although more robust CD8+ T cells infiltrated the area of the tumor compared with primary tumors, infiltration of CD4+ T cell subsets was not observed. The results in this trial may indicate the importance of tumor-specific Th1 responses for immunotherapy to be effective. In other words, the limited success of the DC-glioma fusion cell vaccine may have particular relevance to the failure of tumor-specific Th1 induction and/or the existence of tolerogenic CD4+ T cell subsets.

6.2. Intratumoral injection of DCs

Intratumorally injected DCs can acquire and process tumor antigens in situ, migrate via lymphoid vessels to regional lymphoid organs, and initiate significant tumor-specific immune responses, even in the CNS (112, 113). It has been reported that DCs have antigen capturing, processing and trafficking abilities only during their immature state (114). In this context, Yamanaka and colleagues reported the results of a phase I/II clinical trial of DC-based immunotherapy for glioma patients in which 5 patients (1 with anaplastic astrocytoma and 4 with GBM) received intradermal administration of tumor lysate-pulsed DCs, and 5 patients (1 with glioma, 1 with anaplastic mixed glioma and 3 with GBM) received intratumoral administration of immature DCs with intradermal administration of tumor lysate-pulsed DCs (115). In their report, shrinkage of contrast-enhanced lesions was observed in neuro-images in the intradermal plus intratumoral administration group, indicating that intratumorally injected immature DCs may have the potential to induce an anti-tumor immune response by capturing and processing in situ TAAs. Based on this result, intratumoral injection of DCs may be a promising strategy for recurrent glioma patients or for patients when a surgical tumor specimen is not available, although the effect of glioma cells to suppress endogenous APC function should not be neglected.

6.3. Subsequent chemotherapy

Wheeler et al. reported that therapeutic DC-based vaccination synergizes with subsequent chemotherapy to elicit tangible clinical benefits for GBM patients (116). In their report, survival and progression times were analyzed retrospectively in 25 vaccinated (13 with and 12 without subsequent chemotherapy) and 13 non-vaccinated GBM patients. Vaccinated patients receiving subsequent chemotherapy exhibited significantly longer times to tumor recurrence after chemotherapy relative to their own previous recurrence times, as well as significantly longer post-chemotherapy recurrence times and survival relative to patients receiving vaccination alone or chemotherapy alone. Patients exhibiting objective (>50%) tumor regression, extremely rare in GBM, were also confined to the group of vaccine with chemotherapy. Given the already described ability of DC-based immunotherapy to induce TAA-specific immune responses and the functional implication of TAAs to generate refractory activity to chemotherapy (117, 118), it is likely that the elimination of the TAA-expressing glioma cell population by the immune response resulted in increased sensitivity to chemotherapeutic drugs in the residual immune-refractory population. Based on this concept, the series use of rational multiple modalities that target different tumor characteristics may be required for the successful treatment of these tumors.

7. CONCLUSIONS

The CNS is not an immunologically tolerated site. It creates a privileged site that regulates the infiltration, activation, and recruitment of peripherally circulating leukocytes. Despite such a peculiar microenvironment that hinders the operation of the immune system, it is now recognized that clearance of transformed glioma cells is a routine physiological function of the normal, non-compromised immune system. Based on the demonstrated ability of DCs to initiate antigen-specific T cell responses and the potential of the CNS to allow activated T cell infiltration, the use of DCs for the treatment of glioma may be the most appropriate method, and the key to the therapeutic success of this strategy is likely to lie in eliciting TAA-specific CTL and Th1 responses. To escape immune surveillance, however, clinically apparent gliomas develop complex mechanisms that suppress tumoridical immune responses by suppressing endogenous APCs, inactivating TAA-specific CTL responses, and by inducing tolerogenic T cell responses. Given these complex and divergent mechanisms by which glioma avoids tumor-specific immune responses, an effective treatment paradigm for malignant gliomas may eventually require a multifaceted approach combining two or more different therapeutic strategies to enhance anti-tumor responses and to reduce the ability of gliomas to suppress immune responses. Such scenarios may involve the use of cytokine therapy, genetically modulated DCs and pharmacological treatments. Additionally, given the heterogeneity of this disease process and an immune-refractory tumor cell population, the series use of rational multiple modalities that target different tumor characteristics may turn out to be the most effective therapeutic strategies for the treatment of malignant gliomas. In this context, subsequent chemotherapy may be one of the most promising rational strategies.

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Immunity in glioma patients with DC vaccine

**Key Words:** Dendritic Cell, T Cell, Central Nervous System, Glioma, Cancer, Glial cell, Immunotherapy, Tolerance, Review

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