Neuro-Oncology

MEETING REPORT

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IMMUNOTHERAPY TASK FORCE FOR MALIGNANT BRAIN TUMORS

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INVITED LECTURE

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The first Immunotherapy Task Force meeting was held in Toronto, Ontario, in conjunction with the national meeting of the American Association of Neurological Surgeons, on April 21, 2001. It was sponsored by the Joint Section of Tumors of the American Association of Neurological Surgeons/Congress of Neurologic Surgeons and the National Institutes of Health. The meeting was organized in response to numerous inquiries by patients, medical physicians, and other neurosurgeons regarding the role of immunotherapy for malignant brain tumors.

The goals of this meeting were 3-fold: to present and evaluate current laboratory and clinical research in the field of brain tumor immunotherapy in order to coordinate and disseminate current information and ongoing research in the field; to try to come to a consensus regarding what clinical end points and immune system monitoring parameters should be evaluated in clinical studies; and to evaluate the need and timing for an Immunotherapy Consortium for conducting multicenter trials, in both clinical and basic science.

This group was a multidisciplinary research group, a kind of "neural environment" for facilitating communication and research. The invitation was open to all interested investigators, and the participants included basic scientists, clinical neurosurgeons, and neuro-oncologists. The meeting was organized in 3 parts: an invited lecture, research talks, and a round-table discussion.

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²Abbreviations used are as follows: CTL, cytotoxic T lymphocyte; DC, dendritic cell; GM-CSF, granulocyte macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; LAK, lymphokine-activated killer; MAb, monoclonal antibody; MHC, major histocompatibility complex; NK, natural killer; TGF, transforming growth factor.

The meeting began with Dr. Darell D. Bigner's inspiring talk that reviewed the basic science and early clinical immunotherapy trials as well as newer and innovative work.

Monoclonal Antibodies

Dr. Bigner acknowledged Dr. Stephen Mahaley for his pioneering work in the 1960s with MAbs² and targeted therapies. Recent clinical trials using MAbs have included therapies such as radioimmunotherapy, which links antibodies to radiation sources. A recently completed phase I trial investigating the use of 131-I-81C6, an iodinated antitenascin MAb injected into the post-operative tumor bed, demonstrated a significant increase in survival in newly diagnosed glioblastoma patients. A phase II study is showing similar results. Another promising area is that of immunotoxin therapy, which links MAbs to a variety of toxins as a means of delivering targeted therapy. One such example, PE38-TP38, was developed by Dr. Ira Pastan and is a *Pseudomonas* exotoxin linked to TGF- α . Such therapies can be delivered in high concentrations directly to the brain tumor via the technique of convection, developed by Dr. Edward H. Oldfield.

Vaccine Approaches

Dr. Bigner discussed his work in the field of tumor vaccines utilizing peptides, anti-idiotypic MAbs, and DC technology. To overcome the problems associated with DCs primed with tumor antigens, including autoimmunity and encephalomyelitis, the novel approach taken by Dr. Bigner and his colleagues is to use an antigen specific for brain tumors as the target for DCs. In particular, the peptide from the mutated epidermal growth factor receptor (EGFRvIII), present on a significant portion of brain tumors, was used as a target in pre-clinical studies. Such studies demonstrated the efficacy and safety of such a treatment strategy with significant potential for improved antitumor immunologic responses.

RESEARCH REPORTS

Thirteen talks were presented. The abstracts follow.

Heterogeneity of Immune Regulation in the CNS

L.A. Lampson, L.P. McCluskey, T. Dutta, Y. Kondo, T. Seabrook, and C. Dube

Advances in many areas contribute to renewed enthusiasm for brain tumor immunotherapy. We point to an area that has not yet received wide attention: heterogeneity in immune regulation within a given tumor and at different sites within the brain. To visualize individual disseminated tumor cells or micrometastases (microtumor), rat tumor cell lines are made to express *E. coli*-derived β -galactosidase (β -gal) or other visualizable markers. To probe heterogeneity in immune regulation, graded doses of IFN- γ or other cytokines are injected into discrete brain regions. Enhanced entry of T cells and activation of antigen-presenting cells are measured. Both T-cell entry and activation of antigen-presenting cells can be enhanced by cytokine injection. The efficiency of IFN- γ action differs in different brain regions. Of interest for brain stem glioma, brain stem responds with high efficiency. Local neurochemicals influence the response to cytokine. The path of drug flow can also be a factor. The response to immune stimulation can vary within a tumor, with microtumor more easily influenced than a large tumor mass. Immunotherapy should take into account the tumor site within the brain. Immunotherapy may be especially effective against microtumor-tumor that cannot be imaged or accessed by other means.

Active Immunotherapy of Human Gliomas With Autologous Tumor Cells Engineered to Block Their TGF- β 2 Secretion

H. Fakhrai, J. Mantil, L. Liu, G. Nicholson, C. Murphy-Satter, G. Kraus, J. Ruppert, and D.L. Shawler

High-grade gliomas secrete elevated levels of TGF- β 2 resulting in immune suppression of patients. TGF- β 2 suppresses induction of the T-cell response and deactivates NK and LAK cells by a feedback mechanism. In a phase I clinical trial, we used autologous tumor cell vaccines that were antisense gene-modified to block TGF- β 2 secretion in patients with either recurrent glioblastoma multiforme or gliosarcoma. Gene modification resulted in a 55% to 98% reduction in TGF- β 2 secretion by the vaccines. Six patients were inoculated 3 to 8 times at 3- to 4-week intervals with 5×10^6 , 1×10^7 , or 2×10^7 autologous TGF- β 2 antisense gene-modified tumor cells. None of the patients had greater than grade 2 toxicities that were not resolved before the next injection. Skin biopsies of the injection sites in patients indicated an endogenous immune response in the form of increased CD4, CD8, and CD68 immune infiltrates after 2 to 5 injections. Tumor biopsy and autopsy data subsequent to treatment showed necrosis of the tumor or prominent lymphocytic infiltrates in the tumor bed in the 4 patients examined. MRIs suggested tumor pro-

gression in 3 patients, a transient response in 1 patient, and tumor necrosis with regression in 2 patients.

Oncolytic Herpes Vectors as an "In situ Vaccine" for Tumor Therapy

R. Martuza

Conditionally replicating viral vectors were initially developed for tumor therapy because of their oncolytic potential. However, in immune competent animals, a second effect is noted and has been termed an "in situ vaccine" effect. As the virus replicates and kills tumor cells, the body develops a robust immune response not only to the replicating virus but also to tumor cell antigens. This response can cause rejection of established tumors at a distance as well as develop lasting immune memory to tumor rechallenge. Moreover, the "in situ vaccine" response can be enhanced through base vector design or through the expression of cytokines or T-cell costimulatory factors. Recent developments in these approaches will be discussed.

DC Immunotherapy and Allogeneic Tumor Cells/Fibroblasts Secreting GM-CSF

J. Yu and K. Black

We demonstrated that vaccination with peptide-pulsed DCs or tumor cells engineered to secrete GM-CSF elicits a T cell-mediated immune response in murine glioma models. Based on these studies, we initiated 2 phase I studies for patients with malignant glioma, the first using DC immunotherapy with tumor lysate derived peptides (15 patients), and the second using a vaccine consisting of allogeneic glioma cells mixed with fibroblasts engineered to secrete GM-CSF (4 patients). Preliminary results demonstrated that both forms of vaccination can induce a cytotoxic T-cell response that targets intracranial tumor cells. Five patients vaccinated with DCs underwent reoperation for recurrent tumor. Three of 5 patients had significant intracranial T-cell infiltration compared with prevaccination. These cells consisted of CD8⁺ cytotoxic T cells and CD45RO⁺ memory T cells. One patient required re-operation after GM-CSF vaccination. Whereas there were significant numbers of CD8⁺ cytotoxic T cells and microglia, there was a paucity of CD45RO⁺ memory T cells postvaccination compared with an absence of these cells prevaccination. NK and B cells were absent before and after vaccination in all cases. Both forms of immunotherapy were well tolerated, and no significant adverse events were noted. Four patients on the dendritic cell study died from tumor recurrence, and autopsy demonstrated a paucity of T cells in these patients. No evidence of autoimmunity was noted. Vaccination with DCs or GM-CSF-secreting cells is safe and elicits a cytotoxic T-cell response associated with memory T cells with DCs and naive T cells with GM-CSF.

Alloreactive CTL for Therapy of Recurrent Brain Tumors

C.A. Kruse, K.R. Winston, N. Foreman, W. Warmath, and K.O. Lillehei

Preclinical studies were performed to test the hypothesis that alloreactive CTLs sensitized to the MHC proteins of the patient offer selective and targeted kill of glioma cells because glioma cells express MHC, which is largely not expressed by normal brain cells. Thus MHC in patients, in effect, acts as a tumor-specific antigen. When adoptively transferred into the CNS, an immunologically semiprivileged site, alloreactive CTLs have certain theoretical advantages over cellular therapy with autologous cells. First, alloreactive CTLs should have the ability to migrate through brain parenchyma to reach individually penetrating tumor cells or pockets of infiltrating tumor and lyse them before they are targeted by the host immune system for destruction. Second, because sensitization is proceeding against major antigen, rather than minor tumor antigen, larger numbers of precursor cells should be available for reaction. Thus, supplying the patient with therapeutically meaningful numbers of effector cells should be possible. Third, use of an allogeneic source of effector cells should overcome possible limitations that might be experienced with immunosuppressed T-helper deficient autologous lymphocytes. A phase I clinical trial was opened in January 1995 at the University of Colorado Health Sciences Center and University Hospital that entailed multiple intracavitary placements of alloreactive CTLs into the resected tumor beds of recurrent glioma patients. Six patients with recurrent malignant glioma (aged 26-46 years) were treated with intracavitary alloreactive CTLs and human recombinant IL-2. Two additional patients, 1 adult (aged 43 years) and 1 pediatric patient (aged 7 years), were treated with alloreactive CTLs by intrathecal placement. Patient lymphocytes that express a high relative antigen density of MHC antigen were irradiated and placed into hollow fiber bioreactors with lymphocytes from MHC-disparate donors. Alloreactive CTLs developed in the presence of low concentration IL-2 over a 2- to 3-week period. The CTLs were phenotypically heterogeneous, but many of the preparations largely expressed CD3 and CD11a/CD8 markers. Targets displaying the human leukocyte antigens of the patient were susceptible to lysis. CTLs were implanted into the tumor bed at surgery, and a catheter was used for subsequent infusions. Patients received from 1 to 5 treatment cycles every other month; 1 cycle consisted of 2 to 3 CTL infusates within a 1- to 2-week period. Different unrelated MHC-disparate donors were used for each cycle. Treatment was well tolerated; transient toxicity at grades 1-3 by National Cancer Institute Common Toxicity Scale criteria were experienced. Three glioblastoma patients died. Two died from local tumor recurrence and the other experienced a recurrence at a site distant from the instillation site of the biologic. Two of 6 patients completed 5 cycles; 1 anaplastic oligodendroglioma patient had recurrent tumor at 32 months from the start of immune therapy.

An anaplastic astrocytoma patient showed stable disease at 75 months from initiation of immune therapy. One anaplastic oligodendroglioma patient, who was removed from protocol during her second treatment cycle, also had no evidence of tumor at 75 months.

IL-4-HSV-TK Autologous Glioma Vaccine for Malignant Gliomas

I.F. Pollack, H. Okada, M.T. Lotze, L.D. Lunsford, D. Kondziolka, F. Lieberman, D. Schiff, J. Attanucci, H. Edington, W.H. Chambers, P. Robbins, J. Baar, D. Kinzler, T. Whiteside, and E. Elder

The use of novel immunotherapeutically based strategies represents a potentially appealing approach for patients with malignant gliomas, provided issues of safety and efficacy can be confirmed. Our ongoing IL4-HSV-TK autologous glioma vaccine protocol represents a direct translation of our extremely promising in vitro results into a therapeutically relevant strategy. After approval of the study by the Protocol Review Committee, Institutional Review Board, Institutional Biosafety Committee, Recombinant Advisory Committee, Food and Drug Administration, and National Cancer Institute, patient recruitment and screening were initiated. Important entry criteria were the presence of a resectable recurrent malignant glioma that could be sufficiently debulked to render the patient steroid independent for at least 2 weeks before vaccination, and the demonstration of intact cell-mediated immunological function. Glioma cells and fibroblasts were then harvested, expanded, and transduced with the IL4-HSV-TK vector, and transduced cells were selected. The patient was then treated with 2 courses of vaccination using IL4-transduced fibroblasts admixed with irradiated autologous tumor cells followed by ganciclovir administration. The initial patient treated on this protocol, a 64-year-old man with a multiply recurrent glioblastoma, exhibited dramatic disease regression, as demonstrated both clinically and radiologically, which first became apparent approximately 3 months after initial vaccine administration. He also exhibited evidence of a productive immunological response at the tumor vaccine site as assessed by histologic and reverse transcriptase-polymerase chain reaction analysis, as well as serological immunity against his tumor. No signs of experimental allergic encephalitis have been detected. He continued to show progressive improvement 8 months following vaccination, but subsequently developed progressive disease.

The Brain Parenchyma is Permissive for Full Antitumor CTL Effector Function

P.R. Walker, T. Calzascia, V. Schnuriger, N. Scamuffa, P. Saas, N. de Tribolet, and P.Y. Dietrich

Effective antitumor immune responses against cerebral malignancies have been demonstrated in several models, but precise cellular function of specific effector cells is poorly understood. We have explored this topic by analyz-

ing the MHC class I-restricted T-cell response elicited after implantation of HLA-CW3-transfected P815 mastocytoma cells (P815-CW3) in syngeneic mice. In this model, tumor-specific CTLs use a distinctive repertoire of T-cell receptors that allow ex vivo assessment of the response by immunophenotyping and T-cell receptor spectratyping. Thus, for the first time in a brain tumor model, we are able to directly visualize ex vivo CTLs specific for a tumor-expressed antigen. Tumor-specific CTLs are detected in the CNS after intracerebral implantation of P815-CW3, together with other inflammatory cells. Moreover, despite observations in other models suggesting that CTLs infiltrating the brain may be functionally compromised and highly dependent upon CD4 T cells, in this syngeneic P815-CW3 model, intracerebral tumors were efficiently rejected, whether or not CD4 T cells were present. This observation correlated with potent ex vivo cytotoxicity of brain-infiltrating CTLs specific for the immunodominant epitope CW3170-179 expressed on P815-CW3 tumor cells.

Regional Low-Dose IL-2 Plus Autologous LAK Cell Therapy for Malignant Brain Tumors: Survival, Eosinophils, and Biologic Effects of a Phase I/II Trial
R.L. Hayes

The median survival for adults with recurrent primary malignant gliomas is 56 weeks after surgery, radiation, and chemotherapy. We have developed an aggressive treatment program that utilizes low doses of IL-2 combined with ex vivo activated LAK cells infused via an indwelling catheter placed into the surgical resection cavity. Autologous leukocytes were collected during a standard 3- to 4-h outpatient leukapheresis procedure, then activated ex vivo for 4 to 5 days with high doses of IL-2. The treatment protocol consisted of two 2-week cycles of therapy over a 6-week period. Patients with stable disease or objective response on follow-up MRI scans were retreated at 3-month intervals. Acute and cumulative IL-2-related toxicities were observed, but limited, and included fever, headache, and transient neurologic irritation. Corticosteroid levels and usage were strictly controlled during immunotherapy, although higher doses were used intermittently to mitigate toxicity. In a similar clinical trial, 10 pediatric patients with disseminated primary posterior fossa tumors were treated with intraventricular IL-2 plus LAK cells. After regional intracranial IL-2 administration, peripheral NK activity increased in most patients. In addition, fresh peripheral mononuclear cells became able to lyse the NK-resistant Daudi target cell line, if 50 IU/ml of IL-2 was present during the 4-h 51-chromium release assay. This peripheral IL-2 inducible killing persisted in one clinically responding patient for over a year. Biologic changes included lymphocytic infiltration, regional eosinophilia, tumor necrosis, and the localized production of IL-2, IFN- γ , and IL-12, demonstrated by in situ hybridization and immunohistochemistry. The changes in cerebrospinal fluid differential counts compared with the pretreatment baseline consisted

of the appearance of eosinophils, and in some cases a progressive increase in eosinophils during the course of therapy. Of the 14 grade 4 adult patients who had sufficient cerebrospinal fluid for evaluation after receiving immunotherapy, 8 showed greater than 10% eosinophils in the Ommaya aspirates at some time point during therapy or follow-up. Regional cerebrospinal fluid eosinophilias were also noted in patients receiving intraventricular immune therapy. One patient presented with a white blood count differential of 77% eosinophils in the aspirate fluid, which persisted throughout the treatment course. This regional eosinophilia was confirmed in 3 of 5 of the other patients. However, only 2 of 6 of the patients demonstrated an increase in peripheral eosinophils (33% and 19%, respectively). This finding was in contrast to the adult trial, in which we noted regional intracavitary and tissue eosinophilia, but did not observe an effect on peripheral eosinophil numbers. IL-2 plus autogeneic LAK cells can be safely administered intracavitarily to treat high-grade primary brain tumors with limited toxicity within the CNS. In the phase II trial, 6 of 28 patients had long-term survival of greater than 2 years post reoperation plus immunotherapy, with 2 patients alive more than 8 years. The presence of a marked regional eosinophilia appeared to correlate with increased survival and may be predictive of a biologic and therapeutic response.

Treatment of Primary and Metastatic Brain Tumors Using Immuno-Gene Therapy

R.P. Glick, T. Lichtor, P. Deshmukh, R. Moser, and E.P. Cohen

The prognosis for patients with either a primary or metastatic brain tumor is poor. We developed a novel form of treatment for primary and metastatic brain tumors using allogeneic fibroblasts genetically engineered to secrete cytokines as a cellular vaccine. We used a number of ways to augment the immune system in the development of the vaccine. To prepare the vaccine, genes were transduced into an allogeneic fibroblast cell line that caused the cell to produce cytokines. These cells were subsequently injected into the intracerebral tumor bed that resulted in the development of an antitumor immune response. We investigated the effects of this cellular vaccine on the survival, the specific immunocytotoxic response, and the histopathology of animals with intracerebral tumors. Mice with a primary intracerebral glioma, melanoma, or breast cancer treated with this allogeneic cytokine-secreting vaccine survived significantly longer than did untreated mice. A specific antitumor immune response as measured by chromium-release assay was demonstrated. Histopathology revealed lymphocytic infiltration and smaller tumors in the treated animals. Toxicity studies demonstrated a limited survival of the vaccine in the CNS with no adverse neurologic effects. Long-term survivors, when rechallenged with tumor, demonstrated immune memory. In summary, these results point toward

the use of immuno-gene therapy as a promising new targeted therapy for patients with malignant tumors.

Treatment of Glioblastoma Multiforme With Autologous DCs: A Phase I Study
L. Liao

DCs are antigen-presenting cells specialized for the induction of primary T-cell responses. In this pilot study, we report the preliminary results of a phase I trial of autologous peptide-pulsed DC immunotherapy for patients with malignant glioma. Patients with glioblastoma multiforme were immunized with a series of 3 biweekly intradermal injections on an outpatient basis. Cohorts of 3 to 6 patients received escalating doses of autologous DCs pulsed with constant amounts of acid-eluted autologous glioblastoma surface peptides. Patients were observed for at least 12 weeks after their last vaccination to assess for toxicity and immunological end points. Blood samples were obtained at days -14, 35, and 112 from first immunization to evaluate for antitumor cellular immune responses using standard AlamarBlue T-cell cytotoxicity assays. Brain MRI scans were obtained every 8 weeks to assess for radiologic evidence of clinical responses. Six glioblastoma patients have been treated to date. Two dose levels of autologous peptide-pulsed DCs (1,000,000 cells/injection and 5,000,000 cells/injection) have been evaluated so far, with no treatment-related National Cancer Institute grade 3 or 4 toxicities observed. No physical signs of experimental allergic encephalomyelitis were detected in any of the patients. All patients developed measurable antitumor CTL responses, although there was no significant difference in the magnitude of CTL responses between the 2 dose cohorts tested. There was no MRI evidence of objective clinical response, although 5 of the 6 treated patients had stable disease (with a follow-up of 3 to 18 months). These preliminary results of our phase I study indicate that treatment of glioblastoma multiforme patients with autologous DCs is a safe and promising approach to the treatment of CNS tumors. We are encouraged by the minimal toxicity of this outpatient vaccination strategy. Further phase II studies are necessary to demonstrate clinical effectiveness and impact on survival in brain tumor patients.

Immunosuppressive Treatments Facilitate Propagation Within Tumors by Oncolytic Virus
H. Wakimoto, K. Ikeda, E. Tyminski, and E.A. Chiocca

Recombinant herpes simplex viruses that selectively replicate in tumor cells are being used as novel anticancer agents in preclinical and clinical trials. The capacity of such viruses to selectively propagate inside infected tumors is critical to their efficacy and differentiates them from all other therapies. We have reported that cyclophosphamide treatment increased intraneoplastic propagation of an oncolytic herpes simplex virus in an athymic rat model of

multiple human gliomas. Additionally, complement depletion augments tumor infectivity by virus by suppression of classical complement responses. To clarify the role of cyclophosphamide in facilitating oncolytic virus propagation within tumors, hrR3, an oncolytic herpes simplex virus that has a deletion of ICP6 by the insertion of the *lacZ* marker gene, was injected into 9L gliosarcoma brain tumors in Fischer rats. Three days later, brains were removed for histochemical analysis of *lacZ* expression. While injection with high titer vector (4×10^7 plaque-forming units) gave an initial increase at 3 days in transduced tumor area in animals pretreated with intraperitoneal cyclophosphamide, this increase was transient. This failure of oncolytic virus' spread even in the presence of cyclophosphamide is likely due to the low expression of receptors for herpes virus entry on 9L cells. We thus generated 9L/HveC cells that stably express human herpesvirus entry mediator C (nectin-1). These cells are highly infectible by the oncolytic herpes simplex virus. In fact, cyclophosphamide pretreatment of rats augmented tumor cell-to-tumor cell viral propagation to a much greater extent than that observed in parental 9L tumors. Cyclophosphamide maintained its positive effect on propagation: Three days after initial infection with 1×10^7 plaque-forming units, 80% of 9L/HveC tumor area was transduced after cyclophosphamide pretreatment, while less than 20% was transduced in the absence of cyclophosphamide. The similar augmented transduction within tumor masses was also observed when MGH-I, a double-mutated neuroattenuated oncolytic virus, was used with cyclophosphamide. In an attempt to explore the mechanisms of cyclophosphamide's action, hrR3 was injected intravascularly in Fischer rats. Reverse transcriptase-polymerase chain reaction analysis of cytokine responses revealed that after cyclophosphamide administration peripheral blood mononuclear cells displayed impaired expression of several cytokines, i.e., IFN α , IL-15, and IL-18, that have been reported to have anti-herpes simplex virus functions. Interestingly, IFN- γ response was found to be rather exaggerated by the cyclophosphamide pretreatment, which may be due to macrophage and NK cell responses to actively replicating virus in tumor. Cyclophosphamide enhances oncolytic herpes simplex virus propagation and transduction within tumors in immunocompetent rats. This may be mediated by decreases in elicitation of antiviral cytokines. These findings implicate a potential benefit of transient immunosuppression in the clinical application of oncolytic cancer therapy.

Adoptive Immunotherapy in Patients With Recurrent Glioma: Autologous Whole-Tumor Vaccine + GM-CSF and Adoptive Transfer of Anti-CD-3 Activated Lymphocytes
A.E. Sloan and G. Wood

This trial was designed to determine the ability of autologous whole tumor cell vaccines to induce cell-medi-

ated immune responses in patients with recurrent malignant glioma, as well as to determine whether combining such vaccination with adoptive transfer of in vitro-activated T lymphocytes prolongs survival. Nineteen patients with recurrent malignant glioma who had previously failed external beam radiotherapy and at least one course of chemotherapy were vaccinated twice with irradiated autologous whole tumor cells using GM-CSF as an adjuvant. Patients then underwent leukapheresis followed by adoptive transfer of peripheral blood lymphocytes activated in vitro with anti-CD3 and IL-2. In vivo immune response, radiological response, clinical outcome, and survival were monitored. Seventeen patients developed a delayed type hypersensitivity response to vaccination, which appeared to be directed against the autologous tumor. Eight patients had evidence of a radiologic response and 5 had evidence of clinical improvement. Median survival was 12 months (range, 6-28 months), and both the delayed type hypersensitivity response and the radiological response correlated with survival ($P < 0.02$ and $P < 0.04$, respectively). These preliminary results suggest that autologous whole tumor cell vaccines induce a cell-mediated immune response, which appears to be tumor specific in most patients. Furthermore, vaccination combined with adoptive immunotherapy with in vitro-activated cells may induce radiologic response and improved survival despite advanced disease and immunosuppression.

Immunotherapeutic Responses in Animal Models

A. Parsa, J. Chi, R. Anderson, and J. Bruce

Intracranial rat glioma models are useful for evaluating the efficacy of immunotherapy protocols. Several models have been described using Wistar rats (C6 cell line) and Fischer rats (9L, T9, F98, RG2/D74, RT-2, CNS-1 cell lines). In this report we describe artifacts associated with these models that can potentially be interpreted as an immunotherapeutic response. Growth patterns and immune responses of C6 cells implanted into the brain and flank of Wistar rats were analyzed and compared with an immunogenic syngeneic model (9L/Fischer). To evaluate changes in a syngeneic system, 9L tumor cells were transfected with a supernatant from vector-producing cell lines containing either pNC-TK-Neo or pNC-Neo. After clonal expansion in selective media, cells were confirmed by Northern analysis to express the *TK* gene and/or *Neo* genes. Fischer rats were challenged with either subcutaneous flank or intracranial parenchymal tumor challenge. Retrovirally transfected clones were compared with wild-type cells by measuring nodule size for flank injections on a daily basis or long-term survival after intracranial injections. Parallel controls for each animal were used to assess immunologic infiltrates using antibodies to CD4 and CD8 T-cell antigens. Flow cytometric analysis with MHC-I antibody was used to evaluate upregulation of MHC-I on 9L cells after retroviral transfection.

Wistar rats with C6 tumors developed a potent humoral and cellular immune response to the tumor. Wistar rats given simultaneous flank and intracerebral tumors had a survival rate of 100% compared with an 11% survival rate in control animals receiving only intracranial C6 cells. In a syngeneic system, retrovirally transfected clones showed significant increase in cell-surface expression of MHC-I as measured by flow cytometry. Animals injected with wild-type 9L cells had flank nodule growth steadily until sacrifice was required. In contrast, retrovirally transfected 9L cells grew more slowly, and in some cases regressed completely. Significant differences were also found in long-term survival or animals challenged intracranially ($20 \text{ days} \pm 3.5$ for wild-type 9L compared with 38 ± 4.0 days for retroviral transfected clones; $P < 0.05$; t test). There was a pronounced CD8⁺ T-cell infiltration in flank and brain samples' retroviral clones but not in postimplantation day-matched control wild-type tumor specimens. The C6 glioma induces a vigorous immune response in Wistar rats secondary to its inherent allogenicity. Efficacy of immunotherapy within the C6/Wistar model must be cautiously interpreted. Our results suggest that MHC-I upregulation after retroviral transfection facilitates a potent CD8⁺ T-cell infiltrate that abrogates cell growth in peripheral and central locations.

ROUND-TABLE DISCUSSION

Of the many issues and discussions that arose during the course of the presentations, 2 major questions were further discussed in detail: What clinical monitoring parameters and standard end points are pertinent for clinical immunotherapy trials? Where do we go from here—is there a need for consortium multicenter trials or future conferences?

Clinical Monitoring and End Points for Clinical Trials

To evaluate the efficacy of clinical studies, as well as to communicate the results to patients, standard clinical end points are needed so the results of a particular type of therapy can be compared with another (so we are not comparing “apples and oranges”). The group came to an informal consensus that clinical studies and investigational reports need to include, at the least, information on the following end points when reporting their results:

- Radiographic assessment of the tumor size before and after treatment;
- Functional status of the patient (Karnofsky Performance Scale/neurologic);
- Survival (including overall time and time to progression);
- Assessment of systemic/peripheral immune responses (e.g., ELISpot, chromium release);
- Assessment of immune responses in the CNS (e.g., immunohistochemistry of the tumors for immune

markers or infiltrating lymphocyte immunocytotoxic responses).

Where to Go From Here

The question of the need and timing of an immunotherapy consortium for conducting clinical and basic science studies was raised. This may be a future (or near future) goal because at this time most clinical studies are single-institution phase I studies. When we are clinically ready for multicenter trials, the need for a consortium would be greater. On the other hand, the development of a focused consortium for conducting immunotherapy studies may help investigative groups obtain Food and Drug Administration/Recombinant Advisory Committee approval and necessary funding for performance of these studies. In addition, a consor-

tium may also ensure that standard monitoring parameters required by the Food and Drug Administration and appropriate clinical end points are adhered to in these studies.

Finally, the entire group felt that future conferences were needed in 6-month or yearly intervals. The group expressed the need to share new information, discuss ongoing work, and evaluate results of current studies to foster communication and collaboration among investigators as well as to gather clinical information for patients with brain tumors.

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