Treatment of Brain Tumors Using DNA-Based Vaccines

Terry Lichtor*

Department of Neurological Surgery, Rush University Medical Center, 1725 West Harrison Street, Suite 1115, Chicago, Illinois 60612, USA

Abstract: Antigenic differences between normal and malignant cells of the cancer patient form the rationale for clinical immunotherapeutic strategies. Because the antigenic phenotype of neoplastic cells varies widely among different cells within the same malignant cell-population, immunization with a vaccine that stimulates immunity to the broad array of tumor antigens expressed by the cancer cells is likely to be more efficacious than immunization with a vaccine for a single antigen. A vaccine prepared by transfer of DNA from the tumor into a highly immunogenic cell line can encompass the array of tumor antigens that characterize the patient's neoplasm. Poorly immunogenic tumor antigens, characteristic of malignant cells, can become strongly antigenic if they are expressed by highly immunogenic cells. A DNA-based vaccine was prepared by transfer of genomic DNA from a breast cancer that arose spontaneously in a C3H/He mouse into a highly immunogenic mouse fibroblast cell line, where genes specifying tumor-antigens were expressed. The fibroblasts were modified in advance of DNA-transfer to secrete an immune augmenting cytokine and to express allogeneic MHC class I-determinants. In an animal model of breast cancer metastatic to the brain, introduction of the vaccine directly into the tumor bed stimulated a systemic cellular anti-tumor immune response measured by two independent *in vitro* assays and prolonged the lives of the tumor-bearing mice. Furthermore, using antibodies against the various T-cell subsets, it was determined that the systemic cellular anti-tumor immunity was mediated by $CD8^+$, $CD4^+$ and NK/LAK cells. In addition an enrichment strategy has also been developed to increase the proportion of immunotherapeutic cells in the vaccine which has resulted in the development of enhanced anti-tumor immunity. Finally regulatory T cells ($CD4^+CD25^+$Fox $p3^+$-positive) were found to be relatively deficient in the spleen cells from the tumor-bearing mice injected intracerebrally with the enriched vaccine. The application of DNA-based genomic vaccines for the treatment of a variety of brain tumors is being explored.

Key Words: Gene therapy, brain tumors, tumor vaccine, cDNA, vaccine enrichment, IL-2, regulatory T cells.

INTRODUCTION

Treatment Limitations of Patients with Malignant Brain Tumors

Although technical advances have resulted in marked improvements in the ability to diagnose and surgically treat primary brain tumors, the incidence and mortality rates of these tumors are increasing [1]. Particularly affected are young adults and the elderly. Primary malignant brain tumors are the second leading cause of death in people under the age of 35. Furthermore in the elderly population, mortality rates from these tumors have increased more than 5-fold since 1970 [2]. The present standard treatment modalities following surgical resection including cranial irradiation and systemic or local chemotherapy each have serious adverse side effects. The few long-term survivors are inevitably left with cognitive deficits and other disabilities [3,4]. The difficulties in treating malignant gliomas can be attributed to several factors. Glial tumors are inherently resistant to radiation and standard cytotoxic chemotherapies [5,6]. The existence of blood-brain and blood-tumor barriers impedes drug delivery to the tumor and adjacent brain infiltrated with tumor. Finally the low therapeutic index between tumor sensitivity and toxicity to normal brain severely limits the ability to systemically deliver therapeutic doses of drugs to the tumor.

Transfer of Genomic DNA from One Cell Type to Another Alters Both the Genotype and the Phenotype of Cells that Take up the Exogenous DNA

Classic studies indicate that transfection of genomic DNA from one cell type to another results in integration of the transferred DNA and stable alteration of the genotype of the recipient cells. The transferred genes are replicated as the cells divide and are expressed. Wigler, *et al*., [7] found that the genome of adenine phosphoribosyltransferase-deficient mouse cells was modified to express the missing enzyme by transfer of DNA from mouse cells whose genome included the gene for the missing enzyme. Analogous findings were observed for membrane-associated determinants. Mendersohn, *et al.*, [8] transferred genomic DNA from human cells into polio virus-receptor-negative mouse cells and the transfected cells expressed the missing receptor. Others [9,10] used this approach to identify genes involved in metastasis.

*Address correspondence to this author at the Department of Neurological Surgery, Rush University Medical Center, 1725 West Harrison Street, Suite 1115, Chicago, Illinois 60612, USA; Tel: 312-942-6628;
Fax: 312-563-3358; E-mail: Terry_Lichtor@rush.edu

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Hsu *et al.* [11] and Kavathas and Herzenberg [12] generated stable transfectants of mouse fibroblasts. The transfected cells expressed human membrane T cell antigens, HLA determinants, and B2-microglobulin. The expression of the transferred human genes by the transfected cells was stable, and long-term (more than six months). The proportion of the transfected mouse cells that expressed the human gene of interest was surprisingly large--in the range of 1/500. The importance of these findings for development of DNA-based tumor vaccines is that the transfer of genomic DNA into cells resulted in the expression of genes specifying missing enzymes, genes controlling cell proliferation and metastasis, and genes specifying membrane associated determinants. An analogous approach can be used to prepare a vaccine for use in patients with malignant gliomas. Genes specifying tumor associated antigens (TAAs) that fail to provoke anti-tumor immunity can become highly immunogenic antigenic determinants if they are expressed by highly immunogenic cells.

Multiple Mutant/Dysregulated Genes in Cancer Cells Specify TAAs

A major rationale for the use of DNA-transfer to prepare vaccines for use in cancer therapy is that the vaccine expresses an array of multiple altered genes which define the malignant phenotype. Genetic instability in cancer cells is responsible for the formation of TAAs. TAAs such as β-catenin [13], gp100, Melan A/Mart-1 and tyrosinase in melanoma [14] are differentiation antigens whose expression is dysregulated in cancer cells. Mutant genes also specify TAAs [15, 16]. For example Boon found that a point mutation in a gene in P815 murine mastocytoma cells specified a tumor-rejection antigen. Thus, the malignant cell-population is characterized by the presence of numerous TAAs, some of which are unique and others are differentially expressed by cancer cells but all are strong potential targets of immune-mediated attack.

DNA from the Patient's Neoplasm is the Ideal Source of Tumor Antigens for Immunotherapy

Since the total number of different TAAs within the population of malignant cells is large and diverse, successful therapy will depend upon the use of a vaccine that is capable of inducing immunity to the broad array of tumor antigens that characterizes the patient's cancer. Therapy based on the induction of immunity to a single antigen, or peptide, is less likely to be successful. Multi-epitope vaccines are expected to be more efficacious than single-epitope vaccines. This is especially the case for malignant astrocytomas, where clinically relevant TAAs, i.e., immunity to TAAs that leads to tumor rejection, have not been identified.

Characteristics of the Modified Cell Line Used as the Recipient of Tumor DNA

Among other advantages of this approach, the cells chosen as DNA-recipients can be selected for their ability to enhance the immune response. The expression of both syngeneic and allogeneic MHC-determinants by the DNA recipient cells is important in order to obtain an optimum anti-tumor response [17]. The syngeneic determinants provide a restriction element for direct presentation of TAAs to CTLs of the host. Allogeneic antigens served as potent immune adjuvants. Numerous investigators found that the immunogenic properties of cancer cells could be enhanced if the cells were modified to express allogeneic MHC-determinants [18-23]. The modified cells, which ordinarily proliferate in syngeneic immunocompetent recipients, were recognized as "foreign" and were rejected. In the mouse, immunization with tumor cells altered by the introduction and expression of allogeneic class I genes led to immune-mediated rejection of the malignant cells and the induction of protective anti-tumor immunity. However, the introduction of genes specifying allogeneic determinants into cells from a primary neoplasm is technically challenging and not always successful. In contrast, transfer of DNA from the tumor into highly immunogenic syngeneic/allogeneic cells is consistently and reliably achieved.

Important Advantages of Preparing a Vaccine by Transfer of DNA from the Patient's Neoplasm Into Nonmalignant Fibroblasts

A vaccine prepared by transfer of DNA from the patient's neoplasm into highly immunogenic, nonmalignant human fibroblasts has a number of important advantages. A major advantage is that the cells used as recipients of the DNA can be selected for special properties, which will enhance the anti-tumor immune response. Since the recipient cells are capable of prolonged proliferation *in vitro*, and the transferred DNA is replicated as the cells divide, only a small quantity of DNA from the neoplasm is required to generate the vaccine. In addition, the number of transfected fibroblasts can be expanded as needed, to obtain sufficient quantities for repeated immunizations of the cancer patient. The fibroblasts used as DNA-recipients will also express allogeneic class I determinants which is a desirable feature since this leads to an augmented immune response. In addition a cell line derived from the patient's primary neoplasm does not have to be established, which is the case if genes specifying cytokines, allogeneic MHC-determinants, co-stimulatory molecules or other immune-augmenting properties are to be introduced into the autologous tumor cells. The establishment of tumor cell lines, especially cell lines derived from astrocytomas, is technically difficult, often not feasible and may not be representative of the tumor cell population as a whole. Furthermore hybrid cell vaccines prepared by fusion of tumor cells with antigen presenting cells pose similar concerns [24-26]. Immunization with tumor cells modified to secrete immune-augmenting cytokines such as IL-2 and GM-CSF has been investigated and shown to result in the development of generalized MHC-restricted anti-tumor immune responses in animal models. However tumor cells are also a source of immunosuppressive factors, which inhibit the anti-tumor activity of the effector cells [27,28]. The DNA-based vaccines are successful because a full complement of genes is transferred to the recipient cells which results in a robust signal for the development of anti-tumor immune responses.

Advantages of DNA-based Vaccines Relative to Other Types of Vaccines

A number of different vaccination strategies are currently being evaluated [29-34]. The approaches to vaccination with TAAs include those based on: a) defined antigens or antigenic peptides, b) tumor cell lysates or lysate fractions, and

c) whole irradiated tumor cells or apoptotic tumor cell bodies. Clinical trials involving vaccines prepared using TAAs or TAA-derived epitopes presented by APCs or fed to dendritic cells (DCs) have shown some promising results. However, defined antigens have to be identified and purified, a tremendous effort requiring an "antigen discovery" approach. The quantity of purified antigen must be increased, to enable multiple immunizations of the cancer patient. While new TAAs are being discovered, the question of which TAA to be used in the vaccine is uncertain and extensively debated. The heterogeneity of antigen expression in the tumor cell population is likely to be a concern. Some tumor cells may not express the antigen chosen for therapy. In one study for example, it was found that expression of known tumor antigens such as gp100 and tyrosinase was variable in different melanoma lesions in the same patient [14]. Not all the malignant cells in the patient's neoplasm expressed these determinants. Since the tumor cell population is heterogeneous, tumor cells that fail to express the defined antigen chosen for therapy are likely to escape destruction by the activated immune system.

The major advantage of vaccines prepared by transfer of tumor DNA into nonmalignant fibroblasts is that TAAs do not have to be purified or produced in large quantities. In comparison with protein vaccines, DNA-based vaccines provide prolonged expression and direct presentation of tumor antigens which results in robust and long-lasting activation of the immune system. From a practical point of view, these vaccines are easy and relatively inexpensive to prepare. Unlike other strategies, vaccines can be prepared from only a limited quantity of tumor-derived DNA, which can be obtained from small surgical specimens (vaccines can routinely prepared from 50 μg of DNA). Furthermore, the recipient fibroblasts can be selected to meet the requirement for rapid expansion in culture and MHC restriction. The DNA-based vaccines offer a number of important advantages, which greatly encourage their further development for cancer immunotherapy.

Disadvantages of Transfer of Tumor-Derived DNA Transfer into Fibroblasts for Expression of TAAs

While vaccination based on transfer of tumor-derived DNA into highly immunogenic cells has a number of advantages, there are concerns as well. Since the proportion of total DNA that specifies TAAs is likely to be small, it is possible that a large number of the transfected cells may not express TAAs or may express TAAs at low levels. This concern is minimized, however, by preclinical data which indicate that the proportion of the cells that take-up tumor DNA and express TAAs is sufficient to induce an effective anti-tumor immune response and to significantly increase survival. Another concern related to therapy with DNA-based vaccines is that genes specifying normal "self" antigens are likely to be expressed by the DNA-transfected cells, creating a danger that autoimmune disease might develop, although this has not been observed thus far. Inbred mice immunized with the DNA-based vaccine or tumor-bearing mice injected with therapeutic DNA-based vaccines failed to exhibit adverse effects. Of course, protocols that depend upon the use of tumor cell-extracts, peptide eluates of tumor cells, fusion cells, cDNAs or RNAs derived from tumor cells are subject to the same concern. In DNA-based vaccines, genes encoding determinants expressed by non-neoplastic cells are likely to be present in the largest proportion relative to genes specifying TAAs. While the use of purified tumor antigen in the form of cDNA or polynucleotide vaccines specific for known TAAs eliminates this concern, those types of vaccines are dependent on the selection of the most "relevant" vaccinating epitope, as discussed above. It is also conceivable that a cellular vaccine, including one using nonmalignant fibroblasts might grow in the patient, forming a tumor. Conceivably, a transforming oncogene or a defective tumor suppressor gene might be transferred to a normal cell, provoking a neoplasm although this has not been observed. Overall, the disadvantages of DNA-based vaccines are few and are certainly no more difficult to overcome than those associated with other types of experimental tumor-vaccines.

Defects in TAA Presentation by Tumor Cells

Defects in presentation of TAAs by tumor cells have been described in both murine as well as human tumors [35, 36]. They can result in tumor cell "escape" from host immunity. One mechanism is the loss of MHC determinants, which results in the impaired ability of the tumors to present TAAs. Loss of MHC antigen expression in several murine tumors is correlated with an increase in the malignant properties of the cells [37]. Melanomas that recurred in mice treated with a vaccine prepared by transfer of DNA from murine melanoma cells into mouse fibroblasts were deficient in expression of MHC class I determinants [38]. Primary and especially metastatic cells may have global or selective down-regulation of class I or class II HLA antigens, due to mutations in β2 microglobulin or TAP genes and thus they may fail to present TAAs in an immunogenic form to immune cells. Even if the host generates tumor-specific CTLs, the effector cells may not be able to eliminate the tumor. In addition to a failure to express HLA antigens, tumors may not express co-stimulatory molecules resulting in an inadequate immune response to TAAs by the host. Immunization with a DNA-based vaccine can overcome certain of these tumor "escape" mechanisms.

Significance

The most compelling reason for the vaccination strategy involving DNA-based cellular vaccines is the current lack of effective therapy for patients with malignant gliomas. This is verified by the dismal survival statistics, which have remained essentially unchanged for 30 years. Immunization with a vaccine that induces strong anti-tumor responses is an attractive addition or possibly even an alternative to conventional therapies. The DNA-based vaccines described in this chapter have shown remarkable therapeutic efficiency and survival benefits in some initial murine preclinical studies.

PRECLINICAL EXPERIMENTAL FINDINGS

Treatment of Intracerebral Glioma in C57Bl/6 Mice by Immunization with Allogeneic Cytokine-Secreting Fibroblasts

As an initial study, we measured the survival of C57Bl/6 mice injected intracerebrally (i.c.) with a mixture of Gl261 glioma cells and cytokine secreting LM cells [39]. Gl261

cells are a glioma cell-line of C57Bl/6 mouse origin (H-2^b). LM fibroblasts are derived from C3H/He mice and express H-2^k determinants. We initially evaluated the immunotherapeutic effects of single cytokine-secreting LM-IL-2 cells and double cytokine-secreting LM-IL-2/interferon-γ cells in mice bearing an i.c. glioma. A mixture of G1261 cells and the single or double cytokine-secreting cells were injected i.c. into the right frontal lobe of C57BL/6 mice, syngeneic with G1261 cells. Mice injected i.c. with the mixture of glioma and LM-IL-2 cells survived significantly longer ($P<0.025$) than control mice injected i.c. with an equivalent number of glioma cells alone. Somewhat more dramatic results were obtained for mice injected i.c. with a mixture of glioma cells and LM-IL-2/interferon-γ double cytokine-secreting cells. No prolongation of survival was noted when allogeneic cytokine secreting fibroblasts mixed with tumor cells or tumor antigens were administered subcutaneously in mice with an intracerebral tumor even though a strong anti-tumor immune response was detected in the spleen cells of the treated animals. Of special interest, mice injected i.c. with an equivalent number of LM-IL-2 cells alone lived for more than three months and showed no evidence of ill effects or neurologic deficit. Immunocytotoxic studies demonstrate a significantly elevated cromium release from Gl261 cells co-incubated with spleen cells from mice injected i.c. with glioma cells and the cytokine secreting fibroblasts. This indicates that a systemic anti-tumor response did develop in the mice injected intracerebrally with the cytokine secreting cells in the presence of tumor antigens.

Treatment of Intracerebral Breast Cancer in C3H Mice by Immunization with Syngeneic/Allogeneic Fibroblast Transfected with DNA from Breast Cancer Cells

Whether results obtained by transfer of DNA from a tumor cell line into mouse fibroblasts can be applied to tumors that develop spontaneously is uncertain. Conclusions based on a model system involving tumor cell lines may not apply to neoplasms that arise spontaneously in patients. The appearance of spontaneous breast neoplasms in C3H mice provides an opportunity to investigate this question. DNA isolated from a breast neoplasm that arose in a C3H mouse (H-2^K) was transferred into mouse fibroblasts (H-2^k). To increase their immunogenic properties and to ensure rejection, the fibroblasts were modified to express H-$2K^b$ determinants beforehand. H-$2K^b$ determinants are allogeneic in C3H mice. The results indicated that C3H mice with intracerebral breast cancer treated solely by immunization with fibroblasts transfected with DNA from the same spontaneous breast neoplasm survived significantly longer ($p<0.005$) than mice in various control groups [40].

T Cell Mediated Toxicity Toward Intracerebral Breast Cancer in Mice Immunized with Syngeneic/Allogeneic Transfected Fibroblasts Modified to Secrete IL-2, GM-CSF or IL-18

An MTS cytotoxicity assay was used to detect the presence of T cells reactive with breast cancer cells in mice injected i.c. with the mixture of SB5b cells and the modified, DNA-transfected fibroblasts. The T cells obtained from the spleens of the injected mice were analyzed two weeks after the i.c. injection of the cell mixture. The results indicated that the cytotoxic response of greatest magnitude was in mice injected i.c. with the mixture of SB5b cells and transfected fibroblasts modified to secrete IL-2 or GM-CSF [40]. Lesser cytotoxic effects were present in mice injected i.c. with SB5b cells and transfected fibroblasts modified to secrete IL-18.

The Proportion of T Cells Responsive to Tumor Cells in Mice Bearing an Intracerebral Tumor Immunized Intracerebrally with Syngeneic/Allogeneic Transfected Fibroblasts Modified to Secrete IL-2, IL-18 or IL-2 + IL-18

An ELISPOT-IFN-γ assay was used to determine the proportion of splenic T cells reactive with SB-5b cells in mice immunized with transfected fibroblasts modified to secrete IL-2, IL-18 or both IL-2 and IL-18. The animals were injected i.c. with a mixture of 1.0 X 10^4 SB-5b breast carcinoma cells and 1.0 X 10^6 treatment cells consisting of LMK^bIL-2/SB5b, LMK^bIL-18/SB5b , or a mixture of LMK^bIL-2/SB5b and LMK^bIL-18/SB5b cells. The animals were sacrificed at two weeks and an ELISPOT assay was done using the spleen cells to detect IFN-γ secretion in the presence of SB-5b tumor cells and antibodies against various T-cell subsets. The results indicate that the cellular anti-breast carcinoma immune response was mediated by $CD4^+$, $CD8^+$ and NK/LAK cells [40]. Although IL-18 secreting cells did not produce a significant anti-tumor immune response as detected with the ELISPOT assay, the combination of IL-2 with IL-18 secreting cells did result in an enhancement of the anti-tumor responses in comparison to animals that were treated with IL-2 secreting cells alone.

Increased Numbers of Responding T-cells were Detected in the Spleens and Cervical Lymph Nodes of Naïve Mice or Mice with i.c. Breast Cancer Injected into the Brain with Cells from the Immuno^{high} Pool

An enrichment strategy for the vaccine was developed based on the hypothesis that if aliquots of a transfected cell population were divided into smaller populations, some populations by chance would contain more highly immunogenic cells than others. The populations with higher numbers of immunogenic cells could be identified by their stronger immunogenic response against SB5b cells in C3H/He mice. Two subpools that stimulated immunity to the greatest (immuno^{high} pool) and least (immuno^{low} pool) extents after three rounds of enrichment were selected for further study.

To determine if systemic anti-tumor immunity was generated in tumor-free mice injected i.c. with cells from the immuno^{high} pool, cervical lymph node and spleen cells from the injected mice were analyzed by ELISPOT IFN-γ assays for responding T cells. Naïve C3H/He mice received 2 i.c. injections at weekly intervals of 1.0 X 10^6 cells from the immuno^{high} pool. One week after the second injection, mononuclear cells from the spleens and cervical lymph nodes of the immunized mice were analyzed for the presence of T cells responsive to the breast cancer cells. As controls, an equivalent number of cells from the non-selected master pool or cells from the immuno^{low} pool were substituted for cells from the immuno^{high} pool. As additional controls, the same protocol was followed except that the mice were injected i.c. with equivalent numbers of SB5b cells, with LMK^b cells or with media. Mice injected with SB5b tumor

cells received only one injection. The results from the cervical lymph nodes indicated that the highest number of responding cells was in mice injected i.c. with cells from the immuno^{high} pool ($p < 0.005$ vs. cells from mice in any of the other groups). Similar results were found in studies using the spleen cells from these animals [41].

ELISPOT IFN-γ assays were also used to determine the number of responding T cells in the spleens of mice with i.c. breast cancer injected into the tumor bed with cells from the immuno^{high} pool [41]. A micro cannula was placed into the right frontal lobe of C3H/He mice. SB5b cells (1.0 X 10^4 in 10 μl) were introduced into the brain through the cannula. On days two and nine following, the animals were injected through the cannula into the tumor bed with 1.0 X 10^6 cells from the immuno^{high} pool. As controls, the same procedure was followed except that the cells from the non-enriched master pool or cells from the immuno^{low} pool were substituted for cells from the immuno^{high} pool. As additional controls, the tumor bearing mice were injected into the tumor bed with equivalent numbers of non DNA-transfected LMK^b cells or the mice were injected with SB5b cells alone. The results indicate that the highest number of responding T cells were in the spleens of tumor-bearing mice injected i.c. with cells from the immuno^{high} pool ($p < 0.05$ versus the number of responding spleen cells in mice injected with cells from the master pool and $p < 0.005$ versus the number of spots obtained from any of the other groups).

The effect of antibodies against various T-cell subsets on the cytotoxic response was used to determine the types of cells activated for antitumor immunity in mice injected into the tumor bed with cells from the immuno^{high} pool. The greatest inhibitory effect was obtained when CD4$^+$ antibodies were added to the mixed cell cultures [41]. Lesser effects were observed if the spleen cells were incubated in the media containing CD8$^+$ or NK/LAK antibodies.

T-reg Cells are Relatively Deficient in the Spleens of Mice with i.c. Breast Cancer Injected into the Tumor Bed with Cells from the Immuno^{high} Pool

T-reg cells are potent inhibitors of natural antitumor immunity. The success of immunotherapeutic protocols may depend upon the relative numbers of T-reg cells and cytotoxic T lymphocytes in tumor-bearing animals and patients. Quantitative RT-PCR for Foxp3, a transcription factor characteristic of T-reg cells, was used to determine the relative proportions of T-reg cells in the spleens and brains of mice with i.c. breast cancer injected into the tumor bed with cells from the immuno^{high} pool of transfected cells. Naïve C3H/He mice were injected i.c. with 5.0 X 10^4 SB5b cells along with 1.0 X 10^6 cells from the immuno^{high} pool of transfected cells. One week later, the animals received a second i.c. injection of cells from the immuno^{high} pool through the same burr hole alone. As controls, the same procedure was followed except that the mice were injected with equivalent numbers of SB5b cells and cells from the non-enriched master pool or the immuno^{low} pool. The results indicate that CD4$^+$/CD25$^+$/Foxp3$^+$ T-reg cells were relatively deficient in the spleens but not in the brains of animals injected with cells from the immuno^{high} pool [41]. An analysis by FACS of the spleens of the injected animals revealed a relative deficiency of CD4$^+$/CD25$^+$ T cells and a corresponding increase in the relative numbers of CD8$^+$ cells in the spleens of mice injected i.c. with cells from the immuno^{high} pool.

DISCUSSION

Despite standard therapeutic approaches, the survival of patients with primary or metastatic tumors to the brain has not improved significantly in more than thirty years. There is an urgent need for new and more effective forms of treatment. Immunotherapy, designed to stimulate immunity to the autologous tumor, is under active investigation for a number of different histologic types of cancer. The enhanced immunotherapeutic properties of a vaccine prepared by transfer of a cDNA expression library derived from breast cancer cells into a mouse fibroblast cell line appears to have great potential in treatment of intracerebral tumors. As the transferred cDNA integrates spontaneously into the genome of the recipient cells, replicates as the cells divide and is expressed, the vaccine could be prepared from small amounts of tumor tissue, enabling treatment at an early stage of the disease, when tumor tissue is available in only limited amounts and the tumor is most susceptible to immune-based therapy. However, like other cellular tumor vaccines, only a small proportion of the transfected cell population was expected to have incorporated cDNA fragments that specified tumor antigens. A novel enrichment strategy has also been developed to increase the proportion of immunotherapeutic cells in the vaccine.

A number of different strategies have been attempted to develop vaccines that generate enhanced anti-tumor immune responses in mice and patients with intracerebral neoplasms involving the central nervous system. Vaccines have been prepared by "feeding" antigen presenting (dendritic) cells apoptotic bodies from tumor cells or tumor cell lysates. Introduction of tumor cell-derived RNA into dendritic cells is another approach which has been developed. Immunization with dendritic cells "fed" derivatives of tumor cells or transfected with tumor-RNA can result in the induction of immune responses against the broad array of tumor antigens expressed by the population of malignant cells including tumors of neuroectodermal origin [42, 43]. In patients, immunization with autologous dendritic cells transfected with mRNA from malignant glioma elicited tumor-specific CD8$^+$ cytotoxic T-lymphocyte (CTL) responses against the patient's malignant cells [44]. Although results of dendritic cell immunotherapy have demonstrated promise in animal models, clinical trials have been disappointing thus far [43].

Other tumor vaccination strategies have been used including modification of neoplastic cells to generate anti-tumor immune responses. Immunization with tumor cells modified to secrete immune-augmenting cytokines such as IL-2 and GM-CSF has resulted in the development of generalized MHC-restricted anti-tumor immune responses in animal models [36, 45-53]. Selective tumor regression was observed in experimental animals and patients receiving immunotherapy alone, in support of the potential of this type of treatment for patients with malignant disease [54]. The effects of cytokine expression by central nervous system tumors (CNS) were examined initially using glioma cells that were engineered to secrete IL-4 [55]. In these studies it was demonstrated that IL-4 transduced glioma cells resulted

in the development of anti-tumor immune responses. Delivery of an IFN-β expression plasmid by cationic liposomes to the CNS tumor site was also found to induce significant anti-CNS tumor immunity in pre-clinical models [56]. Use of a high-titer adenoviral vector encoding IL-12 is another strategy that was reported to induce anti-tumor responses in a glioma model [57].

Previous studies indicated that transfection of genomic DNA from the malignant cells into a fibroblast cell line resulted in stable integration and expression of the transferred DNA. Both the genotype and the phenotype of the cells that took up the exogenous DNA were altered as portions of the transferred DNA were expressed. Immunization of tumor-bearing mice with the DNA-based vaccine resulted in the induction of cell mediated immunity directed toward the type of tumor from which the DNA was obtained, and prolongation of survival, consistent with the expression of an array of TAA by the transfected cells. This was the case for mice with melanoma, squamous cell carcinoma and in mice with breast cancer [58]. Multiple undefined genes specifying TAA that characterize the malignant cell population were expressed by cells that took up DNA from the tumor. The number of vaccine cells could be expanded as required for multiple immunizations. In addition, the recipient cells can also be modified before DNA-transfer to increase their immunogenic properties, as for example, by the introduction of genes specifying immune-augmenting cytokines or allogeneic MHC-determinants, which act as strong immune adjuvants. In animal models, injection of cytokine-secreting allogeneic fibroblasts into the tumor bed of intracerebral neoplasms was partially effective in the treatment of mice with established brain tumors [59].

To be successful, every remaining tumor cell in the patient must be eliminated. It is unlikely that a single form of therapy is capable of achieving this goal. However immunotherapy in combination with surgery, radiation therapy and chemotherapy will likely find a place as a new and important means of treatment for patients with brain tumors. A major advantage of DNA-based vaccines is that they do not require protein purification or its production and yet they are able to elicit robust and long-lasting activation of the immune response, which results in tumor rejection. From a practical point of view, these vaccines are easy to prepare and they are relatively inexpensive. Only a limited quantity of tumor-derived DNA is required, which can be obtained from small surgical specimens. The enrichment strategy enables the generation of highly immunogenic pools of transfected cells with enhanced immunotherapeutic properties.

Thus DNA-based vaccines offer a number of advantages, which greatly encourage their further development for cancer immunotherapy in general and specifically for treatment of malignant brain tumors.

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