Epidermal Growth Factor Receptor VIII Peptide Vaccination Is Efficacious against Established Intracerebral Tumors

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ABSTRACT

Purpose: The epidermal growth factor receptor (EGFR) is often amplified and structurally rearranged in malignant gliomas and other tumors such as breast and lung, with the most common mutation being EGFRvIII. In the study described here, we tested in mouse models a vaccine consisting of a peptide encompassing the tumor-specific mutated segment of EGFRvIII (PEP-3) conjugated to keyhole limpet hemocyanin [KLH (PEP-3-KLH)].

Experimental Design: C57BL/6J or C3H mice were vaccinated with PEP-3-KLH and subsequently challenged either s.c. or intracerebrally with a syngeneic melanoma cell line stably transfected with a murine homologue of EGFRvIII. Control mice were vaccinated with KLH. To test its effect on established tumors, C3H mice were also challenged intracerebrally and subsequently vaccinated with PEP-3-KLH.

Results: S.c. tumors developed in all of the C57BL/6J mice vaccinated with KLH in Freund’s adjuvant, and there were no long-term survivors. Palpable tumors never developed in 70% of the PEP-3-KLH-vaccinated mice. In the C57BL/6J mice receiving the PEP-3-KLH vaccine, the tumors that did develop were significantly smaller than those in the control group (P < 0.05). PEP-3-KLH vaccination did not result in significant cytotoxic responses in standard cytotoxicity assays; however, antibody titers against PEP-3 were enhanced. The passive transfer of sera from the immunized mice to nonimmunized mice protected 31% of the mice from tumor development (P < 0.05). In vivo depletion studies showed that the effector cell population was natural killer and CD8+ T cells, and in vitro assays showed that macrophages could lyse target tumor cells with serum from the PEP-3-KLH-vaccinated mice. Peptide vaccination was also sufficiently potent to have marked efficacy against intracerebral tumors, resulting in a >173% increase in median survival time, with 80% of the C3H mice achieving long-term survival (P = 0.014). In addition, C3H mice with established intracerebral tumor that received a single treatment of PEP-3-KLH showed a 26% increase in median survival time, with 40% long-term survival (P = 0.007).

Conclusions: Vaccination with an EGFRvIII-specific peptide is efficacious against both s.c. and established intracerebral tumors. The therapeutic effect of peptide vaccination may be mediated, in part, by antibody-dependent cellular cytotoxicity.

INTRODUCTION

The EGFR4 gene is often amplified and mutated in human neoplasms (1, 2). The most common mutation, EGFRvIII, enhances tumorigenicity (3–5) and occurs in a large proportion of malignant brain tumors (1) and tumors that commonly metastasize to the brain, such as breast and non-small cell lung carcinomas (6). The mutation is characterized by a consistent in-frame deletion of 801 bp from the extracellular domain that splits a codon and produces a novel glycine amino acid at the fusion junction (1, 2). This fusion junction encodes a tumor-specific protein sequence that is expressed on the surface of tumor cells but is not present in normal tissues, making it an ideal target for antitumor immunotherapy.

The specific targeting of therapeutic agents to tumors has been a goal of cancer therapy ever Ehrlich first suggested it almost a century ago (7). Although the induction of autoimmunity might be desirable in nonessential tissues (such as the prostate) infiltrated with tumor, autoimmune encephalomyelitis could be a lethal consequence of this approach in patients with primary brain tumors. Indeed, the induction of a lethal autoimmune reaction in the CNS of nonhuman primates given adjuvant has already been demonstrated convincingly (8). The ability to

1 The abbreviations used are: EGFR, epidermal growth factor receptor; CNS, central nervous system; mAb, monoclonal antibody; ADCC, antibody-dependent cellular cytotoxicity; KLH, keyhole limpet hemocyanin; GM-CSF, granulocyte macrophage colony-stimulating factor; DPBS, Dulbecco’s PBS; NK, natural killer.
target immunotherapy to a tumor-specific antigen such as EGFRvIII should therefore reduce the possibility of inducing deleterious autoimmunity.

The novel EGFRvIII epitope has previously been exploited for a variety of immunotherapies, including the intratumoral injection of mAb (9) and as a target of dendritic cells (10). A peptide vaccine that can be synthesized easily and made commercially available would be an attractive approach to immunotherapy. Already such peptide vaccinations have been applied to systemic cancers.

To determine whether a peptide vaccine directed to the splice junction of EGFRvIII might be effective in the treatment of extracerebral and intracerebral tumors, we vaccinated mice with an EGFRvIII peptide. This proved to be highly effective against s.c. and established intracerebral syngeneic tumors. We have shown previously that antibodies directed against the EGFRvIII injected directly into an intracerebral tumor can mediate ADCC. In the current study, we found that a systemic peptide vaccine directed against the EGFRvIII produces humoral responses that may also be mediating ADCC.

MATERIALS AND METHODS

PEP-3. PEP-3, a 13-amino acid peptide with a terminal cysteine (LEEKKGNYVVTDHC) that spans the EGFRvIII mutation, was synthesized at AnaSpec, Inc. (San Jose, CA). The purity of the peptide preparation was >95%, as assessed by high-pressure liquid chromatography. The peptide was conjugated to KLH at a 1:1 ratio (w/w) and used for immunization. The immunogens were diluted in distilled H2O to a concentration of 2 mg/ml.

Vaccination Strategy. We administered 100 μg of the immunogen in a 1:1 ratio with Freund’s complete adjuvant 8 weeks before the administration of tumor cells either s.c. (C57BL/6J mice) or intracerebrally (C3H mice or C57BL/6J mice). This was followed by the administration of 100 μg of the immunogen in Freund’s incomplete adjuvant at 6 and 2 weeks before the tumor challenge. These vaccinations were administered in the base of the tail, right groin, and left groin, respectively. The dose of GM-CSF administered during each vaccination was 800 units/ml in a final volume of 100 μl. In the intracerebral treatment experiments, the C3H mice also received 100 μg of the immunogen in a 1:1 ratio with Freund’s complete adjuvant 4 days after tumor challenge. The negative control vaccination consisted of KLH mixed 1:1 with Freund’s complete adjuvant administered 8 weeks before tumor challenge and followed by KLH mixed 1:1 with Freund’s incomplete adjuvant at 6 and 2 weeks before tumor challenge.

Tumor Cell Lines and Murine Models. The B16/F10 murine melanoma cell line was derived from a spontaneous melanoma in the C57BL/6J mouse of the H-2B background and was provided by Isaiah Fidler (The University of Texas M. D. Anderson Cancer Center, Houston, TX). K1735 is a murine melanoma cell line syngeneic in the C3H background and was also provided by Isaiah Fidler. These cell lines were stably transfected with a murine homologue of the human EGFRvIII as described previously (9-11). The GM-CSF-transfected B16 cell line has been described previously (11). The cell lines were grown in zinc-option medium (Life Technologies, Inc., Gaithersburg, MD) containing 10% (v/v) fetal bovine serum. All cell lines were grown in antibiotic-free medium and free from Mycoplasma contamination (12).

In the in vivo experiments, we used 4-6-week-old, strictly inbred C3H or C57BL/6J mice (The Jackson Laboratory, Bar Harbor, ME) maintained in the Duke University Cancer Center Isolation Facility (Durham, NC) in accordance with Laboratory Animal Resources Commission standards.

Tumor Formation. To induce the s.c. tumors, logarithmically growing B16EGFRvIII cells were injected into the right hind flank of C57BL/6J mice at a dose of 1.5 × 10^6 cells suspended in 100 μl of PBS. Tumors were measured every third day. A mouse was euthanized when it became unable to reach food or water. The mean tumor volumes were calculated on the basis of the tumors that grew in the surviving mice.

To induce intracerebral tumors, either B16EGFRvIII or K1735EGFRvIII cells were collected in logarithmic growth phase, washed twice with PBS, mixed with an equal volume of 10% methyl cellulose in zinc-option medium, and loaded into a 250-μl syringe (Hamilton, Reno, NV) with an attached 25-gauge needle. The needle was positioned 2 mm to the right of the bregma and 4 mm below the surface of the skull at the coronal suture using a stereotactic frame (Kopf Instruments, Tujunga, CA). The intracerebral tumorigenic dose for the K1735EGFRvIII and B16EGFRvIII cells was 2 × 10^5 and 5 × 10^5, respectively, in a total volume of 5 μl.

In Vitro Cytotoxicity Assay. Cytolysis of the B16EGFRvIII cells by splenocytes obtained from non-tumor-bearing C57BL/6J mice 2 weeks after vaccination with either PEP-3-KLH or KLH was measured using standard assays (13). Negative control splenocytes were obtained from naïve C57BL/6J mice. Positive control splenocytes were obtained from C57BL/6J mice vaccinated with B16EGFRvIII cells transfected with GM-CSF as described previously (11). Lysis of the target B16EGFRvIII cells was measured in a 4-h chromium release assay.

For the in vitro macrophage-mediated lysis assays, B16EGFRvIII cells were labeled with ^3H and then incubated on ice in a solution of DPBS containing purified serum dilutions or antibodies. The labeled B16EGFRvIII cells and serum dilutions or antibodies were then added to wells containing peritoneal macrophages harvested from C57BL/6J mice purified by plastic adherence for 2 h. Specific lysis was calculated by subtracting background release induced by macrophages and B16EGFRvIII cells incubated together without serum. The degree of lysis was expressed as a percentage of the maximal possible lysis induced by 1 N NaOH.

PEP-3 ELISA. Serum was obtained from sedated, non-tumor-challenged C57BL/6J mice vaccinated with PEP-3-KLH, PEP-3, or KLH. The serum samples were stored at −20°C before analysis in a PEP-3-specific ELISA. The 96-well ELISA plates were prepared by overnight incubation with 10 μg/ml PEP-3 in carbonate buffer (pH 9.0). Serum antibody binding to PEP-3 was detected with secondary biotinylated goat antibodies specific for mouse IgG, IgG1, or IgG2a antibodies (Amersham Biosciences, Arlington Heights, IL) and observed with streptavidin-alkaline phosphatase (Life Technologies, Inc.) and the color reagent p-nitrophenyl phosphate (Pierce, Rockford, IL). Absorbance was measured at 405 nm with a Titertek Multiscan.
MCC/340 ELISA plate reader (Flow Laboratories, Glasgow, Scotland) using ScanSoft software (Flow Laboratories).

**Serum Transfer.** Serum obtained by sequential bleeds 200 days after vaccination was pooled from each individual donor mouse and stored at −20°C. Unpurified serum was brought to room temperature and administered i.v. to a matched recipient mouse 1 day before tumor challenge and then for 4 consecutive days at a dose of 200 μl/day.

**Leukocyte Subset Depletions.** Granulocytes, CD4+ T cells, CD8+ T cells, or NK cells were depleted in vivo as described previously (13). Anti-CD4 (GK1.5) and anti-CD8 (2.43) murine mAbs were produced as described previously (13). R. Cofman (DNAX, Palo Alto, CA) provided RB6, an antibody used for granulocyte depletion. Polyclonal rabbit anti-asialo GM1 antibody against murine NK cells was obtained commercially (Wako Chemicals, Richmond, VA). All antibodies were injected once i.v. 3 days before the tumor challenge and i.p. every 5 days thereafter with a pretitated amount of the antibody. Flow cytometric analysis of splenocytes with FITC-labeled anti-CD3 (145-2c11), anti-CD4 (GK1.5), and anti-CD8 (53-6.72) antibodies (BD PharMingen, San Diego, CA) confirmed a >97% depletion of the targeted subset and a normal level of other subsets. NK cell depletion was confirmed by immunohistochemical staining of the spleen. Granulocyte depletion was confirmed by analysis of the peripheral blood smears.

**Immunohistochemistry.** Analysis of formalin-fixed paraffin-embedded tissue with a mAb to EGFRvIII (L8A4) was performed on 5-μm-thick sections that were deparaffinized in sequential baths of xylene and 100% ethanol and blocked for endogenous peroxidase activity by incubation in 0.3% H2O2-absolute methanol for 10 min. After rehydration in PBS for 10 min, sections were placed in heat-induced epitope retrieval buffer (BioTec Solutions, Santa Barbara, CA) and microwaved for two 5-min cycles with H2O replacements as necessary. Slides were allowed to cool for 20–30 min, rehydrated in PBS, and then blocked for 30 min at room temperature in 10% normal rabbit serum, followed by exposure to the primary reagent (L8A4 or control IgG1 at 10 and 5 μg/ml or PBS on serial sections) for 2 h at room temperature. Slides were washed in PBS, and the appropriate dilution of biotinylated rabbit anti-mouse IgG (DAKO Corp., Carpenteria, CA) established by previous titration was applied, and the slides were incubated for 30 min at room temperature. Slides were washed again in PBS and exposed to horseradish peroxidase-avidin complex for 30 min, and then exposed to 33 μg/ml 3,3′-diaminobenzidine (DAB) (Metal Enhanced DAB Substrate Kit; Pierce). Positive and negative primary reagents and the tissue control (the EGFR–III–expressing D256 human glioma xenograft) were run with each assay.

**Statistical Analysis.** Long-term survival was defined as more than double the median survival time of KLH-vaccinated or treated mice. The exact permutation Wilcoxon rank-sum test was used to compute the P, and the exact Hodges-Lehmann method was used to compute the median difference in the s.c. tumor volume between groups with corresponding 95% confidence intervals. Survival curves were estimated for each vaccination group using the product-limit estimation of Kaplan and Meier (14). These curves were compared using the Cox proportional hazards regression model. Statistical significance was determined at <0.05. The in vitro cytotoxicity and macrophage lysis data were analyzed using the unpaired standard t test.

**RESULTS**

**Vaccination with PEP-3-KLH Protects Mice against the Formation of EGFRvIII-expressing s.c. Tumor Cells.** To determine whether vaccination with a peptide corresponding to the mutated portion of the EGFRvIII protein (PEP-3) protected against the formation of s.c. tumors in a syngeneic system, C57BL/6J mice were vaccinated three times with PEP-3-KLH, PEP-3, or KLH (n = 10 mice/group/experiment). The figure is a result of a single experiment, but it was replicated twice. B16EGFRvIII cells are B16 murine melanoma cells stably transfected to express a murine homologue of the human tumor-specific EGFR mutation EGFRvIII. In the PEP-3-KLH-vaccinated group, the P for the tumor volume in the PEP-3-KLH-vaccinated group versus the KLH control group was <0.0001 at day 20, 23, 26, 29, and 32 and 0.0020 at day 36. For the PEP-3-vaccinated group, the P was 0.052, 0.0012, 0.0028, 0.0012, 0.0002, and 0.0028 at day 20, 23, 26, 29, 32, and 36, respectively.

![Fig. 1](image-url) Volume of s.c. B16EGFRvIII tumors in C57BL/6J mice vaccinated three times with PEP-3-KLH, PEP-3, or KLH (n = 10 mice/group/experiment). The figure is a result of a single experiment, but it was replicated twice. B16EGFRvIII cells are B16 murine melanoma cells stably transplanted to express a murine homologue of the human tumor-specific EGFR mutation EGFRvIII. In the PEP-3-KLH-vaccinated group, the P for the tumor volume in the PEP-3-KLH-vaccinated group versus the KLH control group was <0.0001 at day 20, 23, 26, 29, and 32 and 0.0020 at day 36. For the PEP-3-vaccinated group, the P was 0.052, 0.0012, 0.0028, 0.0012, 0.0002, and 0.0028 at day 20, 23, 26, 29, 32, and 36, respectively.

**Vaccination with PEP-3-KLH Fails to Induce Cytotoxic Responses.** To determine whether peptide vaccination induced cytotoxic responses that could be detected in standard in vitro cytotoxic assays, splenocytes from the C57BL/6J mice vaccinated with PEP-3-KLH or KLH and from naïve mice were stimulated in vitro with B16EGFRvIII cells, and cytotoxicity was assessed against 51Cr-labeled B16EGFRvIII target cells. The PEP-3-KLH-vaccinated mice, KLH-vaccinated mice, and naïve mice showed no significant lysis of the B16EGFRvIII target cells compared with the positive control mice vaccinated with B16EGFRvIII cells transfected with GM-CSF (data not shown).
Vaccination with PEP-3-KLH Results in Humoral Immunity. To determine whether PEP-3-KLH vaccination resulted in a PEP-3-specific humoral response, PEP-3-KLH-, PEP-3-, and KLH-vaccinated C57BL/6J mice were examined for humoral responses at 2, 4, 6, and 8 weeks after the first vaccination by PEP-3 ELISA. At all of these times, sera from PEP-3- and KLH-vaccinated mice showed little or no humoral response to PEP-3 (<0.001 mg/ml), whereas sera from the PEP-3-KLH-vaccinated mice (n = 10) had concentrations of anti-PEP-3 IgG1 antibodies equivalent to concentrations of a PEP-3-specific mAb ranging from 0.001 to >0.01 mg/ml.

Passive Transfer of Serum from PEP-3-KLH-vaccinated Mice Mediates an Antitumor Response. To determine whether the serum was sufficient to mediate the antitumor response, serum from the vaccinated C57BL/6J mice was passively transferred to recipient C57BL/6J mice 1 day before the s.c. tumor challenge and then for 4 consecutive days thereafter. The C57BL/6J mice receiving serum from naïve (n = 8) or KLH-vaccinated mice (n = 5) all succumbed to s.c. B16EGFRvIII tumors. In contrast, 18% of the mice receiving serum from the PEP-3-KLH-vaccinated mice without tumor challenge (n = 11) failed to develop s.c. B16EGFRvIII tumors. Furthermore, 31% of mice receiving serum from the PEP-3-KLH-vaccinated mice with tumor challenge failed to develop s.c. B16EGFRvIII tumors (n = 13; P < 0.05; Fig. 2).

Depletion of NK or CD8+ T Cells Abrogates the Efficacy of PEP-3-KLH Vaccination. Because vaccination with PEP-3-KLH did not show any significant cytotoxicity but did result in humoral responses that can mediate ADCC, we performed in vivo depletion studies of granulocytes, CD4+ T cells, CD8+ T cells, and NK cells in C57BL/6J mice vaccinated with PEP-3-KLH 3 days before tumor challenge to determine which cell subsets were involved in the effector function. Long-term survival occurred in 70% of the positive control, PEP-3-KLH-vaccinated mice in the absence of cell depletion (n = 10; median survival, >120 days), and this represented a >186% increase in the median survival time compared with that in the KLH-vaccinated mice (n = 10; median survival, 42 days; P = 0.0019). Depletion of CD4+ T cells (n = 10) or granulocytes (n = 4) did not alter the efficacy of the PEP-3-KLH vaccinations. However, depletion of CD8+ T cells (n = 10) diminished the efficacy of the PEP-3-KLH vaccination. Additionally, depletion of the NK cells led to a significant decrease in the efficacy of the PEP-3-KLH vaccinations, with 30% of the mice showing long-term survival and no increase in the median survival time compared with that in the KLH-vaccinated mice (P = 0.44). Furthermore, the efficacy of the PEP-3-KLH vaccination in suppressing tumor volume was abrogated when NK or CD8+ T cells were depleted (Fig. 3).

Macrophages Mediate Lysis of B16EGFRvIII Cells. To determine whether macrophages could also be involved in the effector function of PEP-3-KLH vaccination and because the complete in vivo depletion of macrophages is not possible, we performed in vitro macrophage-mediated lysis assays. In the absence of macrophages, the murine PEP-3-KLH serum induced minimal lysis. Furthermore, in the absence of PEP-3-KLH serum, the macrophages induced minimal lysis. However, in the presence of macrophages, the murine PEP-3-KLH serum propagated significant enhanced EGFRvIII-specific tumor cell cell lysis (P < 0.05; Fig. 4).

Systemic Vaccination with PEP-3-KLH Protects Mice against the Formation of Intracerebral Tumors. To determine whether vaccination with PEP-3-KLH protected against the formation of intracerebral tumors in this model, C57BL/6J mice received three vaccinations, as described in “Materials and Methods,” of either PEP-3-KLH (n = 10) or KLH (n = 10). The C57BL/6J mice then received intracerebral injection with 5 × 10^5 viable B16EGFRvIII cells. In two separate experiments, the PEP-3-KLH- and KLH-vaccinated mice had a median survival time of 24 and 22 days (P = 0.048), respectively (data not shown), indicating that there was significant but minimal benefit of the vaccine against intracerebral tumors in this model.

One potential reason for the PEP-3-KLH vaccination showing suboptimal efficacy against B16EGFRvIII intracerebral tumors was the suboptimal binding of peptides spanning the EGFRvIII mutation to class I MHC in the C57BL/6J background (H-2B) as predicted. However, in the C3H background (H-2K), PEP-3 was predicted to have excellent binding to class I MHC. Therefore, in two separate experiments, C3H mice received three vaccinations of PEP-3-KLH (n = 10) or KLH (n = 10) and then received intracerebral injection with 2 × 10^3 K1735EGFRvIII cells. Vaccination of the mice with PEP-3-KLH or KLH resulted in a median survival time of >120 days and 44 days, respectively (P = 0.014; Fig. 5). The survival time in the PEP-3-KLH-vaccinated mice was >173% longer than that in the KLH-vaccinated mice.

5 http://bimas.dccc.nih.gov/molbio/hla_bind/
Treatment of C3H Mice with Established Intracerebral Tumors with PEP-3-KLH Is Efficacious.

To determine whether treatment was also efficacious against established intracerebral tumors, C3H mice with established intracerebral K1735EGFRvIII tumors were treated with a single dose of PEP-3-KLH in Freund’s complete adjuvant. Forty percent of the mice survived long term, and there was a 26% increase in median survival time in the C3H mice treated with PEP-3-KLH compared with the KLH controls ($n = 10$; $P = 0.0076$; Fig. 6). These results are especially important in light of the paucity of published immunotherapy reports demonstrating efficacy against established intracerebral models.

Vaccination of C3H Mice with PEP-3-KLH and GM-CSF Is Efficacious against Intracerebral Tumors. To determine a clinically useful adjuvant vaccination protocol because CFA is not approved for use in human subjects, C3H mice were vaccinated with PEP-3-KLH with either Freund’s complete adjuvant followed by Freund’s incomplete adjuvant or Freund’s incomplete adjuvant plus GM-CSF at 8, 6, and 2 weeks before the intracerebral injection of tumor cells. Control vaccinations consisting of Freund’s incomplete adjuvant with KLH and GM-CSF or Freund’s complete adjuvant with KLH resulted in a median survival of 44 and 46 days, respectively (Fig. 7). In contrast, C3H mice vaccinated with PEP-3-KLH with either Freund’s complete adjuvant followed by Freund’s incomplete adjuvant or Freund’s incomplete adjuvant plus GM-CSF showed median survival times of 67 and 74 days, respectively ($P < 0.0001$).

Treatment Failures Are Secondary to EGFRvIII-negative Escape Variants. To determine the EGFRvIII status of mice that failed to respond to the PEP-3-KLH vaccination, relapsing s.c. tumors were evaluated by immunohistochemistry. EGFRvIII expression by immunohistochemistry was lost in 80% of relapsing tumors ($n = 5$) after PEP-3-KLH vaccination, indicating that EGFRvIII-negative escape variants were a potential mechanism of treatment failure in active immunotherapy.

DISCUSSION

Using a syngeneic tumor model, we showed that a systemic vaccination consisting of a tumor-specific peptide has potent activity against both s.c. and established intracerebral tumors. The fact that we could obtain significant efficacy in an intracerebral treatment model emphasizes the potency of this approach. Thus far, there has been a paucity of reports published on immunotherapies sufficiently potent to treat established brain tumors (9, 15, 16).

The efficacy of a peptide vaccine targeting the EGFRvIII has been reported previously (17). In the model system used, NIH-3T3 cells were stably transfectected with a human, not a murine, EGFRvIII gene and implanted into outbred NIH Swiss mice. This model system is associated with histoincompatibility problems resulting from the use of nonsyngeneic outbred mice.
and the fact that a xenogeneic human antigen, not a murine homologue, was transfected into an outbred murine model system. These incompatibilities may account for the differences observed in the mechanism of response between our model systems and the previously published report. For example, we did not observe cytotoxic responses using standard cytotoxicity assays in our syngeneic model systems, in contrast to the previously published report, despite the fact that in vivo depletion studies indicated that CD8+ T cells were involved. Interestingly, in a Phase I clinical trial of glioma patients vaccinated with dendritic cells pulsed with PEP-3-KLH and GM-CSF, PEP-3-KLH delayed-type hypersensitivity reactions were observed. Furthermore, the malignant glioma patients had PEP-3-KLH-specific CD4+ T-cell ELISPOT reactions, which would indicate that the PEP-3-KLH is capable of elaborating class II responses as well.

Furthermore, we observed PEP-3-specific humoral responses. In fact, in our passive transfer experiments, serum from the PEP-3-KLH-vaccinated mice protected against the development of tumors. To further investigate the in vivo protection mechanism of PEP-3-KLH vaccination, we performed depletion studies of NK cells to demonstrate the abrogation of the efficacy of the PEP-3-KLH vaccination. The serum resulting from vaccination of mice with PEP-3-KLH was capable of lysing tumor target cells in the presence of macrophages. These combined studies showed that peptide vaccination, in this model system, is capable of inducing ADCC, which may have contributed to the in vivo efficacy we observed in our intracerebral model system.

The use of a systemic peptide vaccination resulting in humoral responses that can mediate clinical efficacy within the “immunologically privileged” CNS has also been convincingly demonstrated in a transgenic Alzheimer’s murine model system (18–20). In this model system, peptide vaccination targeting amyloid results in antibody-mediated phagocytosis by microglia, resulting in the reduction of CNS plaques. The use of a...
peptide vaccine directed against a tumor-specific antigen whose mechanism of efficacy appears to be humorally related is attractive because macrophages (21–24), microglia (25), and astroglial cells (26) all contain Fc receptors, which are abundant in brain tumors and throughout brain tissue. In fact, our laboratory has previously demonstrated the efficacy of humoral responses that target the EGFRvIII antigen in intracerebral tumors (9). In this study, a mAb, Y10, specific to EGFRvIII was directly injected into established intracerebral tumors, and this led to a >488% increase in the median survival time. The underlying mechanism of efficacy was Fc dependent and likely due to ADCC. The advantages of a systemic peptide vaccine for humans with intracerebral tumors include its easy route of administration as opposed to intracerebral delivery of an antibody, its ability to induce potent immunological and antitumor responses, and its easy translation into a commercial product.

Although tumor specificity has obvious advantages, one potential problem with an agent that selectively targets a single tumor-specific mutation is the intrinsic antigenic heterogeneity of the cells in malignant tumors. Although EGFRvIII seems to represent a nearly terminal branch of malignant progression for malignant brain tumors and is expressed clonally within this lineage, this therapy might confer a selective growth advantage on neoplastic cells not expressing this epitope. We have shown previously (9) that treatment failures with antigen-specific passive humoral immunotherapy are not a result of antigen-loss variants. However, EGFRvIII expression by immunohistochemistry was lost in the majority of relapsing tumors after PEP-3-KLH vaccination, indicating that EGFRvIII-negative escape variants were a mechanism of treatment failure in active immunotherapy. This could be potentially overcome in the future with a multiple peptide vaccination approach, especially in light of the emergence of newly identified tumor-specific and tumor-associated antigens.

A second potential problem with a peptide vaccine is suboptimal binding of the peptide in certain patient haplotypes. Based on the range of binding affinities of other tumor-specific peptide, antigens capable of eliciting lymphocyte responses, patients with a haplotype background of HLA-B4403, -B2705, -B5201, -B60, or -B61 would be predicted to have homologous binding affinities to PEP-3-KLH. One of these haplotypes, HLA-B27, is more common in glioma patients at a frequency of 19% compared with 7.5% within the general population (27). Therefore, the overall chance that any given glioma patient would have one of the aforementioned class I haplotypes predicted to have binding to PEP-3-KLH is 64%. Thus, patients most likely to respond to the PEP-3-KLH and GM-CSF vaccine include those with high-grade gliomas and other EGFRvIII-positive metastatic malignancies that are not immunosuppressed and with a MHC complex that optimally binds the peptide.

In summary, we have created a murine homologue of an important human mutation in the EGFR. Within this model, systemic vaccination with a peptide that spans this mutation, potent antitumor activity is generated against s.c. and established intracerebral tumors. This vaccination results in antibody responses that are specific to the EGFR and may be partially responsible for the efficacious responses seen within the CNS.

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