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IL-12 Treatment of Endogenously Arising Murine Brain Tumors

Edward J. Roy,† Ute Gawlick,* Brent A. Orr,* Laurie A. Rund,‡ Andrew G. Webb,‡ and David M. Kranz†

A number of recent studies have indicated that T cells can be stimulated to attack transplanted brain tumors in rodent models. As IL-12 has been shown to activate cytotoxic T cell responses, we tested the idea that it might stimulate a T cell response against endogenous brain tumors that arise in SV40 large T Ag transgenic mice (SV11). SV11 mice develop tumors of the choroid plexus, a specialization of the ependymal lining of the brain ventricles. They are a particularly relevant model of human disease, because they are immunocompetent but immunologically tolerant of the tumors. SV11 mice were treated with recombinant murine IL-12 for 10 days. Tumors grew more slowly than in control treated mice, and in some cases were reduced in size, as assessed by magnetic resonance imaging before and after treatment. At the end of treatment, tumors, but not brain parenchyma, exhibited extensive infiltration of activated CD8⁺ and CD4⁺ T cells. Tumors also showed a reduction in vascular density. Mice treated with IL-12 lived significantly longer than control mice. Tumors that progressed were nearly devoid of T cells, indicating that the T cell response was not sustained. In addition, some mice that had a substantial tumor burden at the beginning of treatment displayed evidence of immunosuppression, which might be related to TGF-β2 detected in tumors. We conclude that IL-12 treatment can initiate an anti-tumor response even against endogenously arising brain tumors, but factors that will allow a sustained and more effective anti-tumor response need to be determined. The Journal of Immunology, 2000, 165: 7293–7299.

Interleukin 12 has shown potent anti-tumor activity in several murine model systems, including melanomas, lymphomas, colon cancer, and mammary cancer (1). In some instances, the anti-tumor activity has been attributed to inhibition of angiogenesis, at least partly mediated by the induction of IFN-γ and inflammatory protein-10 (2, 3). In other instances, a T cell-mediated anti-tumor response has been shown to be dependent on CD4⁺ and CD8⁺ T cells (4, 5). Direct up-regulation of tumor cell MHC by IL-12 could contribute to the T cell-mediated anti-tumor effect (6). Finally, the anti-tumor activity of IL-12 might also depend on the action of NKT cells (7).

IL-12 has shown some effectiveness against brain tumors transplanted into the CNS. Using Rous sarcoma virus-induced astrocytoma cells (RSV-M) transplanted to the subarachnoid space, Kishima (8) showed that IL-12 elicited an anti-tumor response, characterized by infiltrating CD4⁺ and CD8⁺ cells. That the effect was immune cell mediated was suggested by the observation that IL-12 did not provide survival benefit when RSV-M was transplanted into nude mice. Furthermore, Ikuchi (9) found that the anti-tumor effect of IL-12 against another Rous sarcoma virus-induced murine glioma (SR-B10A) was dependent on CD8⁺ cells and required vaccination with IL-2-producing irradiated tumor cells. Interestingly, only intracerebral injection of IL-12- and IL-2-producing cells, not systemic administration, protected animals against growth of the intracerebral tumor. In another murine transplant model, the combination of IL-12 and peripheral vaccination with glioma cells transfected with B7 and ICAM-1 retarded growth of glioma cells in the CNS (10). The rat glioma 9L can be effectively treated with systemic or intracerebral IL-12 (11, 12). However, 9L is a highly immunogenic cell line that elicits a moderate CNS T cell infiltrate even in the absence of treatments (13).

We have characterized a model of an endogenously arising murine brain tumor to determine the potential utility of eliciting T cell-mediated responses against CNS tumors to which the immune system is tolerant or ignorant (14, 15). SV11 mice are transgenic for the large T Ag, which is tumorigenic because it inhibits p53 and pRB. Under the T Ag promoter, transgene expression in the SV11 line results in tumors restricted to the choroid plexus, a specialized region of the ependymal lining of the ventricles of the brain (16). Tevethia (17) showed that SV11 mice are tolerant to tumor Ags, but that splenocytes transferred from C57BL/6 background strain mice to SV11 mice can initiate a response against brain tumor Ags. We have shown that T cells activated by either an anti-CD3 Ab or the superantigen staphylococcal enterotoxin B (SEB)³ can be targeted to the tumor by a bispecific, Ab-ligand conjugate (15, 18). The ligand folic acid binds to high affinity folate receptors that are abundant on the surface of SV11 tumors as well as human choroid plexus tumors and ependymomas (19). Given the finding that IL-12 is able to shift a response of T cells that interact with a peptide from a tolerizing/anergizing condition to an activating condition (20, 21), we hypothesized that IL-12 might be useful in stimulating a T cell response against SV11 tumors.

However, the effectiveness of IL-12 against brain tumors may be affected by the condition of the blood-brain barrier, particularly if IL-12 confers its anti-tumor effects locally at the site of tumors.

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For brain tumors, the type of tumor and the extent of progression may affect the blood-brain barrier. Human choroid plexus tumors and ependymomas show contrast enhancement with gadolinium in magnetic resonance imaging (MRI), indicating a disrupted blood-brain barrier (22, 23). We have determined with Evans Blue tracer studies that in large SV11 tumors the blood-brain barrier is also disrupted (18). In the present experiments we used MRI to examine the accessibility of the tumors before IL-12 treatment at the age of 85 days, when tumors are typically small and asymptomatic. As described below, even small SV11 tumors showed contrast enhancement with gadolinium, suggesting that IL-12 might also gain access to the tumors.

In the present experiments, IL-12 was found to be effective in generating a T cell response, reducing tumor size, and prolonging survival in most mice bearing SV11 tumors. However, when treatment was administered at a later point in the development of the tumors, immunosuppression appeared to occur. Immunosuppression is known to be characteristic of many tumors, particularly brain tumors. Although several factors have been suggested to mediate local and systemic immunosuppression observed in human glioma patients, most evidence indicates that TGF-β is involved (24). Isoforms 1–3 of TGF-β are heterogeneously expressed in individual gliomas, but most appear to express TGF-β1 and TGF-β2 (25). In fact, TGF-β2 was originally isolated as a glioma-derived T cell suppressor factor, and it has been detected directly in tumors, tumor cysts, and CSF of approximately one-third of brain tumor patients, including all ependymomas examined (26). Therefore, we also investigated whether TGF-β1 or TGF-β2 was present in SV11 tumors as a potential variable that may affect responses to IL-12.

Materials and Methods

Mice

SV11 mice heterozygous for the SV40 large T Ag gene on a C57BL/6 background were obtained from Terry Van Dyke (University of North Carolina, Chapel Hill, NC), and SV11+ males were bred with C57BL/6 females to maintain the line. Genotyping was performed by PCR for the T Ag gene. The survival curves of control SV11+ mice in the reported experiments are virtually the same as those originally reported in 1985 (16). Transgene-positive males and females have the same survival curves. Animals were housed in barrier cages in animal facilities of the University of Illinois. All studies were approved by the laboratory animal care advisory committee.

Reagents: cytokines and Abs

Recombinant murine IL-12 was provided by Stan Wolf (Genetics Institute, Cambridge, MA). Rabbit anti-CD3 was obtained from Dako (Carpinteria, CA), rabbit anti-CD3 (PECAM, clone MEC 13.3) was obtained from PharMingen (San Diego, CA), rat anti-CD8α was purchased from Serotec/Harlan (Indianapolis, IN), chicken anti-TGF-β1 was obtained from R&D Systems (Minneapolis, MN), rabbit anti-TGF-β2 was purchased from Santa Cruz Biotechnology (Santa Cruz, CA), biotinylated goat anti-rabbit and biotinylated goat anti-rat mouse adsorbed were obtained from Vector (Burlingame, CA), and biotinylated anti-chicken IgY was purchased from Jackson ImmunoResearch (West Grove, PA).

MRI and IL-12 treatments

At 85 days of age the baseline body weight of mice was determined, and mice were examined for any neurologic signs or indications of hydrocephalus. Animals (<10% of mice) were excluded from experiments if there was evident cranial expansion due to a large tumor. Some mice were anestheitized with 100 mg/kg ketamine/15 mg/kg xylazine and imaged using a 1.5-Tesla, 33-cm bore magnet (Oxford Instruments, Oxford, U.K.) with actively shielded gradients. Pulse programming and data acquisition were performed on an Apollo spectrometer (Tecmag, Houston, TX). A six-turn solenoid (diameter, 1.8 cm; length, 1.8 cm) was used for transmission and reception. Data acquisition parameters were as follows: spin-echo pulse sequence with repetition time, 0.5 s; echo time, 30 ms; two signal averages; data matrix, 128 × 128 complex data points; 10 slices with full-width half-maximum thickness, 800 μm; and gap between adjacent slices, 600 μm. The total data acquisition time was 6 min. Mice were imaged before and after 50 nmol of gadolinium diethylenetriaminepenta-acetic acid was administered ip.

Treatment with IL-12 or vehicle began on day 85 and continued until day 95. IL-12 (0.1 or 0.5 μg) was injected i.p. daily in 0.1 ml of sterile PBS-1% normal mouse serum vehicle. In one experiment mice were given a single injection of 50 μg SEB on day 84 to provide an initial activating stimulus for T cells. Some cohorts of mice were euthanized on day 95 to determine tumor status at the end of the treatment period, and other cohorts of mice were monitored until they died or were euthanized according to a body weight criterion or neurological impairment. An objective criterion for morbidity was established with previous cohorts of mice by correlating the loss of body weight due to cachexia and age of death. Mice died within 2–3 days of reaching 75% of baseline body weight, so this criterion was used to determine the time of death; mice were also euthanized if they showed early neurological symptoms such as vestibular perisylvian syndrome from fourth ventricle tumors) or ataxia and lethargy. In addition, four mice with evident cranial expansion from the tumors on day 85 were treated with IL-12. These mice were euthanized on days 93–95 when they met the above criteria and were analyzed separately because it was suspected, from spleen weight, that they had failed to respond to IL-12. Additional mice were imaged by MRI on day 85 and again at the end of treatment on day 95.

Postmortem analysis of tumors

Brains were imaged in Cryomatrix (Shandon/Lipshutt, Pittsburgh, PA), snap-frozen in a dry ice/ethanol slurry or isopentane/liquid nitrogen, and stored at −80°C. Spleens were weighed and snap-frozen. Ten-micrometer sections were cut on a cryostat, and every 20th section was saved for staining. Adjacent 10-μm sections were counterstained with 1% methylene green and hematoxylin. Sections were postfixed for 5 s in acetic acid/zinc/formalin (Newcomer Supply, Middleton, WI) for CD3 and CD8 staining, for 3 min in acetic acid/zinc/formalin for TGF-β, or for 3 min in glyoxal/ethanol (Prep-tech, Anatech, Battle Creek, MI) for CD3 staining, rinsed, dried, and stored at −80°C. Sections were washed with PBS/0.1% Tween, endogenous peroxide was quenched with 3% H2O2, and sections were blocked with Superblock (Pierce, Rockford, IL). Primary Ab incubation was overnight at 4°C in a humid chamber. Secondary Ab was incubated at room temperature for 2 h, followed by either avidin-biotin-HRP (ABC HRP Elite, Vector) or tyramide signal amplification with streptavidin-HRP (New England Nuclear, Boston, MA). Chromogen was nickel-cobalt-enhanced diaminobenzidine (DAB; Pierce) or NovaRed (Vector). For TGF-β immunohistochemistry, slides were subjected to microwave buffer. A 1 h reaction was performed using either avidin-biotin-HRP (ABC HRP Elite, Vector) or tyramide signal amplification. Detection of TGF-β was performed with either anti-CD3 or hematoxylin at ×200 magnification using NIH Image. T cells were counted using a grid at ×200 magnification in a random section midway through the tumor; intravascular T cells were not counted. Vascular area was determined in sections stained with either anti-CD31 or hematoxylin at ×200 magnification using NIH Image.

Flow cytometry

After 10 days of treatment, tumors were dissected, weighed, incubated in nonenzymatic cell dissociation solution (Sigma) for 15 min at 37°C, and subsequently passed through a 50-μm mesh nylon filter. Cells were washed twice in PBS containing 0.01% BSA (Sigma). Tumor tissue cell suspensions were incubated with Cyochrome-labeled anti-mouse CD8α (PharMingen) and FITC-labeled anti-mouse CD25 (PharMingen), with Cyochrome-labeled anti-mouse CD3ε (PharMingen) and FITC-labeled anti-mouse CD25, with PE-labeled anti-mouse CD4 (PharMingen), and FITC-labeled anti-mouse CD25, or with isotype-matched control Abs (PharMingen). Cells were analyzed using a Coulter EPICS XL instrument (Hialeah, FL). Dead cells were excluded on the basis of either light scatter or propidium iodide-positive staining. Tumor cells were identified through staining with a fluorescein analogue of folic acid, N-[tert-octyl-(4'-fluoroscin-thiocarbamoyl)-1-yl]-4'-lysine (27). Data were gated by light scatter on the region that contained viable nontumor cells (folate receptor negative and propidium iodide negative). The percentages of CD3+ and CD25+, CD4+ and CD25+, or CD8+ and CD25+ double-positive cells were determined.

Western blot analysis

Cells from five SV11 tumors were pooled, dissociated with nonenzymatic cell dissociation buffer (Sigma-Aldrich, St. Louis, MO), washed free of lysed RBC, extracted overnight with acid/ethanol in the presence of protease inhibitors, precipitated with ethanol/ether, and resuspended and dialyzed in PBS as previously described (28). Extracts (10 μg protein) were
run on 12% SDS gels under nonreducing conditions. The proteins were transferred to nitrocellulose and probed using TGF-β2 or TGF-β1 primary Ab, biotinylated secondary Ab, and streptavidin-HRP. As a control, the extract was immunoprecipitated with TGF-β2 Ab and protein G-Sepharose beads.

**Statistical analysis**

Infiltration data, tumor volume, and tumor vascularity were analyzed by ANOVA using SAS JMP software (SAS Institute, Cary, NC). Survival data were analyzed by Kaplan-Meier estimates and the log-rank test, using SAS JMP software.

**Results**

**MRI analysis of access of blood-borne substances to tumors**

MRI images of mouse brains before and after gadolinium injection clearly showed that substances that do not cross the blood-brain barrier have access to the tumors at 85 days of age (Fig. 1). MRI images of nontumor-bearing mouse brains did not show any gadolinium in the ventricles or parenchyma (data not shown). Tumors smaller than 1 mm in diameter were visible with gadolinium enhancement. Both small and large tumors showed contrast enhancement following gadolinium staining.

**T cell infiltration after 10 days of IL-12 treatment**

In the absence of any treatment, SV11 tumors were nearly devoid of T cells (Fig. 2, A and C). In contrast, daily treatment with 0.1 μg/day of IL-12 from days 85–95 induced a vigorous T cell response (Fig. 2, B and C; p < 0.01, control vs IL-12). T cells were found throughout the tumors, but were rarely observed in brain parenchyma. Staining with anti-CD8 indicated that ~65% of the T cells were CD8+ (Fig. 2C). Analysis of additional animals euthanized after shorter durations of treatment indicated a gradual rise in the number of infiltrating T cells beginning after about 4 days of treatment (data not shown).

Tumors from additional animals treated daily with IL-12 were analyzed by flow cytometry (Fig. 2D). Both CD4+ and CD8+ T cells were present in IL-12-treated tumors. Similar to the immunohistochemical analysis, flow cytometry indicated that 64 ± 5% of the CD3-positive cells were CD8 positive. In IL-12-treated mice, more than half of the CD3-positive cells were also CD25 positive. In IL-12-treated mice, there was also a population of CD25-positive, but CD3-negative, cells (Fig. 2D). In treated mice double-positive CD3+/CD25+ cells comprised 22% of the gated cells compared with 2% in control mice. There was an ~30-fold increase in CD8+/CD25+ cells in IL-12-treated tumors compared with controls (p < 0.01).

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**FIGURE 1.** Lack of a blood-brain barrier in SV11 tumors. MRI images of SV11 tumor on day 85 immediately before and after gadolinium dithiobisphenylazide administered i.p., indicating that the tumors are accessible to blood-borne agents and are not protected by a blood-brain barrier. Tumors <1 mm in diameter are readily detected by MRI (indicated by the arrows). The plane of section is horizontal, with the rostral end to the left. Two small tumors are evident in the lateral ventricles following gadolinium treatment.

**FIGURE 2.** IL-12 induced T cell infiltration of SV11 tumors. A and B, Immunohistochemistry of CD3+ T cells. Mice were treated beginning on day 85 for 10 days with 0.1 μg of murine IL-12 or vehicle. Mice were euthanized, and brains were snap-frozen, cryosectioned at 10 μm, and post-fixed in acetic acid formalin zinc. T cells were assessed with anti-CD3 staining and visualized with nickel-cobalt DAB. A, Tumors from control mice were essentially devoid of T cells. B, IL-12-treated mice showed a strong T cell infiltrate, confined to the tumor and not present in normal brain parenchyma (at the bottom of the figure). C, Quantification of immunohistochemistry of tumor-infiltrating T cells in IL-12-treated and control mice. After brains were processed for CD3 immunohistochemistry, all stained cells were counted in randomly selected sections at a magnification of ×200. Density is expressed as number of T cells per square millimeter; area was determined using NIH Image. Adjacent sections were stained for CD8, and cells were counted. IL-12 treatment was significantly different from control treatment (p < 0.001). D, Flow cytometric analysis of T cell infiltrate in SV11 tumors following IL-12 treatment. Tumors from control and IL-12-treated mice were nonenzymatically dissociated; incubated with Abs for CD3, CD8, and CD25; and analyzed by two-color flow cytometry. Data were gated by light scatter on the region that contained viable non-tumor cells (folate receptor negative and propidium iodide negative). Treatment with IL-12 increased the percentage of double-labeled CD8+CD25+ cells and CD3+CD25+ cells in the tumor compared with control treatment (p < 0.01).
Tumor volume after 10 days of IL-12 treatment

At 95 days the tumor volume of untreated mice was heterogeneous, with an average volume of 107 ± 31 mm³ (n = 9; Fig. 3). This represents ~25% of the total volume of the mouse brain. In contrast, tumors of animals treated with IL-12 for 10 days were reduced in volume by >90%, with an average volume of 9 ± 3 mm³ (n = 6; p < 0.05 vs control; Fig. 3). An additional group of animals received IL-12 and a single injection of the superantigen SEB 1 day before the onset of IL-12 treatment. The volume of tumors in these animals was similar to that in the group treated with IL-12 alone (12 ± 4 mm³; n = 5; p < 0.05 vs control). Comparison of all IL-12-treated animals with untreated control animals was highly significant (p < 0.01). The tumors of several additional mice were imaged with MRI before the onset of treatment and again at the end of 10 days of treatment. To validate volume estimates by MRI, selected mice were imaged, the brains were processed for histology, and stereological estimates of tumor volume were made. Volume estimates of reconstructed hematoxylin- and eosin-stained postmortem sections vs MRI estimates were virtually identical; the correlation of the two types of volume estimates for eight tumors was r = 0.98 (p < 0.001). As assessed by MRI, control tumors increased in volume an average of 503 ± 141%, whereas IL-12-treated tumors that were present at the beginning of treatment actually decreased in volume by 20 ± 14%. In one IL-12-treated mouse, two lateral ventricle tumors were reduced in volume, but a separate fourth ventricle tumor not evident on day 85 was prominent on day 95 (Fig. 4).

Effect of IL-12 on tumor vascularity

Because IL-12 has been shown to inhibit angiogenesis, tumor vascularity was assessed by measuring the cross-sectional area of capillaries in tumors from IL-12-treated and control mice at the end of 10 days of treatment. IL-12 resulted in a 63% reduction in vascular area (Fig. 5; p < 0.01).

Survival of mice treated with IL-12

Mice treated with either 0.1 or 0.5 µg of IL-12/day survived significantly longer than control mice (Fig. 6; p < 0.01 for each comparison). The median survival time of controls was 100 days compared with 120 days for animals given 0.1 µg of IL-12 and 126 days for animals given 0.5 µg of IL-12.

T cell infiltrate after tumor progression

Tumors were analyzed by immunohistochemistry for CD3⁺ T cells after animals were euthanized. There were high levels of CD3⁺ T cells present at the end of IL-12 treatment on day 95 (Fig. 7). In contrast, nearer to the time of death (~120 days of age for IL-12-treated animals) there were few T cells remaining in tumors. Control animals euthanized when moribund at ~100 days of age had very low levels of T cells, similar to untreated controls on day 95.

Spleen hypertrophy in response to IL-12

At the end of 10 days of treatment with 0.1 µg of IL-12, marked spleen hypertrophy was evident in most animals. Spleen weight was increased by over 600% in IL-12-responsive mice compared with untreated controls (Fig. 8; p < 0.01). However, four mice that had evident cranial expansion at the beginning of treatment due to a large tumor showed no spleen hypertrophy after the 10-day IL-12 treatment regimen (Fig. 8). Tumors from these mice were also examined by immunohistochemistry for the presence of T cells in response to IL-12 treatment, and there were only low levels of T cells evident in the tumors (Fig. 7).

TGF-β expression in tumors

As TGF-β1 and TGF-β2 have been implicated in immunosuppression by tumors, we investigated whether SV11 tumors express either isoform of TGF-β. Western blot analysis of tumor cells dispersed and separated from platelets revealed the presence of TGF-β2 (Fig. 9A), but not TGF-β1 (data not shown). Immunohistochemistry of TGF-β1 and TGF-β2 confirmed the Western blot findings, with TGF-β2 being evident especially in capillary endothelial cells of the tumor and scattered in the extracellular matrix.
FIGURE 5. Vascular area following IL-12 treatment. A, Sections (10 μm) of control and IL-12-treated tumors were stained for CD31 (PECAM-1) and hematoxylin, and the cross-sectional area was determined using NIH Image. Results are expressed as the percentage of tumor area occupied by vascular area. Tumors from IL-12-treated mice were significantly less densely vascularized than control tumors (p < 0.05). B, Examples of the degree of vascularity in control and IL-12-treated tumors stained for CD31.

FIGURE 6. IL-12 treatment prolonged the survival of SV11 mice. Mice were treated from days 85–95 with 0.1 or 0.5 μg of IL-12 per day, and controls were treated with PBS-0.1% normal mouse serum vehicle. Mice were considered moribund and were euthanized when they reached 75% of their baseline body weight or when they showed severe neurological deficits. The median survival time was 100 days for controls, 120 days for the mice given 0.1 μg of IL-12, and 126 days for the mice given 0.5 μg of IL-12. Both IL-12 treatments significantly prolonged survival compared with controls (p < 0.05).

FIGURE 7. Tumor-infiltrating T cells after tumor progression and in nonresponding mice. Brains from mice in survival experiments that eventually succumbed to their tumors were processed for immunohistochemistry of CD3 to stain T cells. All stained cells were counted in randomly selected sections at a magnification of ×200. Density is expressed as the number of T cells per square millimeter; area was determined using NIH Image. Nonresponding mice were mice that had evident cranial expansion at the beginning of treatment on day 85 and no increase in spleen weight on days 93–95; these mice had little or no T cell infiltration. Similarly, IL-12-treated moribund mice euthanized at an average of 120 days had few or no T cells remaining in the tumors.

Discussion

The present findings extend the potential therapeutic utility of IL-12 to an endogenously arising solid tumor within the CNS. A treatment regimen of 10 days of IL-12 generated a strong T cell infiltrate, a reduction in tumor vascularity, a substantial reduction in tumor volume, and a significant prolongation of survival. MRI analysis before treatment compared with MRI analysis at the end of treatment indicated that some tumors were reduced in size in response to IL-12 treatment. The T cells that infiltrated the tumors were both CD4+ and CD8+. They were activated, as evidenced by up-regulation of CD25, the receptor for IL-2. At present we do not know whether the T cells were specific for a particular tumor Ag, or whether they accumulated because of more nonspecific activation and migratory factors (29). However, the fact that normal brain tissue was completely free of T cells may indicate that the T cells are specific for tumor-associated Ag(s). The observation that within a given animal some tumors were reduced in volume while others grew may also be consistent with a specific T cell response. SV11 mice usually generate several tumor foci in the choroid plexus, and it is not known how the individual tumors, probably generated from mutations subsequent to p53 and pRB inhibition, differ phenotypically. It is possible that low levels of Ag presentation are occurring in SV11 mice, but T cells become tolerant rather than reactive to tumor Ags. Previous studies have shown that IL-12 can shift a T cell response from tolerance to immunizing for CD8+ T cells in vivo (20, 21).

Tevethia (17) has shown previously that C57BL/6 mice will respond to large T Ag epitopes, and SV11 mice irradiated and reconstituted with C57BL/6 splenocytes can mount an effective anti-tumor response, with mice surviving for >200 days. However, in the present experiments the T cell response was not adequately maintained after treatment was discontinued, and at the time of death the tumors were nearly devoid of T cells. This finding is consistent with other observations. Speiser (30) reported that pancreatic tumors in mice transgenic for SV40 under the insulin promoter could be temporarily controlled by vaccination with a

TGF-β1 was not consistently detectable by immunohistochemistry (data not shown).
model tumor Ag, but that an immune response was not maintained even though tumor cells continued to express MHC I and continued to be susceptible to T cell-mediated killing. Similarly, Mescher (31) reported that an initial control of thymoma tumors expressing an OVA peptide as a model Ag was not maintained by adoptively transferred OVA-specific T cells, because T cells migrated out of the area of the tumor. The brain may present additional problems, as Knopf (32) has reported that in response to minor histoincompatible PS11 mastocytoma transplants into the brain, BALB/c mice generate a population of noncytolytic precursors of CTLs that infiltrate the tumors. The authors suggested that the brain microenvironment may prevent the T cells from developing into fully activated T cells (32).

Part of that microenvironment in the present model may be active inhibition of T cells by TGF-β secreted by the tumor and the tumor vasculature. IL-12 elicited a strong T cell response, except in cases that had already progressed to a large tumor burden before treatment began. In most of these cases splenic hypertrophy and T cell infiltration into the tumor were completely absent. The results suggest that if the immune response is not vigorously maintained, the tumor may progress to the point where immunosuppressive substances such as TGF completely prevent nascent T cell responses. Although TGF-β is one candidate in the present model and in human brain tumors, other data indicate that in gliomas there are additional, non-TGF factors that are immunosuppressive as well (33).

A potential side effect of IL-12 in the CNS is the elicitation of autoimmunity. In this respect, IL-12 has been shown to be involved in relapses of experimental autoimmune encephalitis. In the present experiments we saw no evidence of an autoimmune response in nontumor-bearing mice. However, it remains to be determined whether autoimmunity might become a problem with a more vigorous and sustained T cell response in the CNS.

We are aware of only one other endogenously arising tumor model that has been treated with IL-12, mice transgenic for HER-2/neu (34). Such mice treated prophylactically with IL-12 had a delayed onset of tumor development and T cell infiltration of the tumors, but tumors later progressed, similar to the findings of the present study. These results contrast with the complete responses and T cell memory that are common in transplant models treated with IL-12 (12, 35–37).

The present findings indicate that IL-12 treatment has potential in the treatment of endogenously arising brain tumors, but that a better understanding of the limitations of responses initiated by IL-12 is required. An effective response may require that the tumor be actively growing but not large enough to generate strong immunosuppression. The factors that determine whether a T cell response is maintained have not been characterized in this or other models (30, 31), but the present study provides a tractable model for approaching these important issues.

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