Efficacy of Tumor-Infiltrating Lymphocytes in the Treatment of Hepatic Metastases Arising From Transgenic Intraocular Tumors in Mice

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Purpose. The purpose of this study was to determine if tumor-infiltrating lymphocytes (TIL) isolated from transgenic intraocular tumors were capable of preventing the development of spontaneous hepatic metastases.

Methods. Tumor-infiltrating lymphocytes were isolated from intraocular tumors in immunocompetent mice and were examined for cytolytic activity in vitro. The antigenic phenotypes of the TIL were determined by in situ immunohistology. Cultured TIL were transferred adoptively to immunoincompetent, intraocular, tumor-bearing recipients to determine the efficacy of TIL in preventing spontaneous liver metastases.

Results. Cultured TIL displayed remarkable cytolytic activity in vitro and antitumoral properties after adoptive transfer into immunoincompetent, athymic nude mice and anti-CD4/CD8-treated euthymic FVB/N mice. Hepatic metastases developed in only 2 of 10 athymic nude mice that subsequently received TIL, whereas metastases developed in all 10 control mice. Similar results were found in anti-CD4/CD8-treated FVB/N mice. Hepatic metastases did not develop in any of the 10 mice treated with TIL, whereas extensive metastatic foci developed in all 10 untreated FVB/N control mice. The efficacy of TIL therapy was manifested also by significant prolongation of host survival times.


Tumors of the uveal tract remain the most common primary intraocular malignancies in adults. Although primary uveal melanoma can be treated successfully by enucleation, approximately 50% of patients with uveal tumors die from metastatic disease. The liver is the primary organ involved in melanoma that has metastasized from the uveal tract, and hepatic metastasis is the leading cause of death in patients with uveal melanoma. Thus, preventing and treating the metastasis of uveal melanoma to the liver should lead to a marked improvement in the prognosis of patients with uveal melanoma.

The discovery of tumor-specific antigens on human and murine melanomas stimulated interest in immunologic modalities for controlling the spread of various cancers. Early studies using biologic response modifiers, such as interleukin-2 (IL-2), suggested that activation of immune effector elements could limit the spread of murine melanomas. Subsequent investigations demonstrated that lymphokine-activated killer (LAK) cells were effective in preventing the metastasis of murine B16 melanomas. Adoptive immunotherapy using LAK cells plus IL-2 has yielded positive results in experimental tumor models and in clinical trials. Recent investigations aimed at developing immunotherapeutic protocols that were more effective than LAK–IL-2 therapy have focused on tumor-infiltrating lymphocytes (TIL) isolated from solid tumors and expanded in vitro. Murine studies have shown that TIL are 50 to 100 times more potent than LAK cells in the treatment of solid tumors in vivo. Moreover, some data indicate that TIL hold promise as agents for controlling pulmonary and hepatic metastases.
Although the feasibility of using TIL for treating metastases arising from extraocular tumors has been established, little is known about the antimitastatic properties of TIL isolated from intraocular tumors. The intraocular environment is endowed with unique immunologic characteristics that prevent the expression of various categories of immunologic responses and, thus, could complicate the application of TIL as antimitastatic modalities.\(^\text{19,20}\) Ksander et al\(^\text{21}\) showed that lymphocytes infiltrated intraocular P815 mastocytomas but did not undergo terminal differentiation and were unable to reject the intraocular tumor allografts. However, in another murine intraocular tumor model that used an ultraviolet light-induced syngeneic fibrosarcoma, TIL displayed impressive tumor-specific cytolyis in vitro and in vivo.\(^\text{22}\) Neither study, however, evaluated the antimitastatic effects of the intraocular TIL.

Because neither mastocytomas nor fibrosarcomas normally occur as primary intraocular tumors, the veracity of these models for evaluating the efficacy of TIL as potential antimitastatic modalities is questionable. Such studies would be most appropriately performed using tumors that arise by in situ transformation within the eyes of experimental laboratory animals. The present study used one of several tumor cell lines isolated from intraocular tumors in transgenic FVB/N mice created by the integration of a recombinant transgene comprised of the tyrosinase promoter and SV40 early-region transforming sequences.\(^\text{23}\) The original tumors arose by in situ transformation of cells at the choroid–retinal pigment epithelium (RPE) interface. The tumors can be orthotopically transplanted into the eyes of athymic nude mice, where they grow and eventually metastasize to the liver, thereby displaying metastatic behavior that parallels human uveal melanoma.\(^\text{23}\) However, after intraocular transplantation in immunocompetent FVB/N hosts, the transgenic tumors become infiltrated with TIL that express tumor-specific cytolytic activity in vitro.\(^\text{24,25}\) Thus, the SV40 transgenic murine tumor model offers a unique opportunity to examine the potential of TIL in controlling metastases arising from intraocular tumors.

The goal of this study was to determine if TIL isolated from orthotopically transplanted uveal–RPE tumors would prevent the development of spontaneous hepatic metastases.

### MATERIALS AND METHODS

#### Mice

A transgenic FVB/N (H-2\(^\text{b}\)) mouse line, designated 124E2, was created by the integration of a recombinant transgene comprised of a tyrosinase promoter and SV40 early-region transforming sequences\(^\text{25}\) and is, in many ways, similar to a murine model of intraocular melanoma described by Mintz and colleagues.\(^\text{26}\) In both transgenic animal models, intraocular tumors arise as multiple hyperplastic foci at the choroid–RPE interface in the posterior regions of the eye. Although the intraocular tumors are comprised of spindle and epithelial elements, the predominant morphologic phenotype is epithelioid.\(^\text{23,25}\) Expression of SV40 transcripts in individual mice was confirmed by reverse transcriptase polymerase chain reaction testing of skin and eyes, as previously described.\(^\text{23}\) Normal syngeneic FVB/N and athymic nude Balb/c (H-2\(^\text{d}\)) mice were purchased from The Jackson Laboratories (Bar Harbor, ME). For some experiments, adult FVB/N mice were immunosuppressed by thymectomy and in vivo treatment with anti-CD4 monoclonal antibody.\(^\text{27}\)

Briefly, thymectomized FVB/N were treated with anti-CD4 monoclonal antibody (IgG\(_{\text{2a}}\) monoclonal antibody secreted by rat hybridoma GK 1.5) and anti-CD8 (IgG\(_{\text{2b}}\), monoclonal antibody secreted by rat hybridoma YTS169.4) administered together intraperitoneally (0.25 mg of each antibody per mouse per injection) on days —5, —1, 0, +7, and +14 relative to intraocular tumor challenge (i.e., day 0). In vivo depletion of the relative T-cell population was confirmed by flow cytometry analysis of spleen cells (see Phenotypic Analysis of Tumor-Infiltrating Lymphocytes). All investigations adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

#### Tumor Cell Line

The 124E2 tumor cell line was derived from a choroid–RPE tumor that arose in a transgenic FVB/N mouse bearing the SV40 oncogene.\(^\text{23}\) This tumor cell line expresses SV40 T antigen, as well as melanoma-associated antigens.\(^\text{23,25}\) Based on their morphologic and antigenic characteristics, the primary tumors that regularly develop in the 124E2 mouse line were considered to be a mixture of uveal melanomas and RPE carcinomas.\(^\text{25}\) Thus, the 124E2 choroid–RPE tumor is not entirely analogous to human uveal melanoma. B16 melanoma cells (C57BL/6 origin) were obtained from American Type Culture Collection (Rockville, MD). Both tumor cell lines were cultured in Dulbecco’s modified Eagle’s medium containing 10% heat-inactivated fetal calf serum, 1% L-glutamine, 1% sodium pyruvate, 1% vitamin solution, and 1% antibiotic-antimycotic solution.

#### Intracamerel Transplantation

A modified quantitative technique for the orthotopic intracamerel transplantation of a precise number of tumor cells into the mouse eye has been described.\(^\text{28}\) Mice were deeply anesthetized with 0.66 mg of ketamine hydrochloride (Vetalar; Parke, Davis, Detroit,
Hepatic Metastasis Model

were enucleated on day 40 after intracameral tumor transplantation. Mice were assigned randomly to one of four groups: group 1 received intraperitoneal injections of 0.5 ml complete RPMI twice daily for 5 days as a control; group 2 received intraperitoneal injections of 10,000 U IL-2 twice daily for 5 days; group 3 received 2 x 10^7 TIL administered intravenously; and group 4 received 2 x 10^7 TIL administered intraperitoneally twice daily for 5 days. For comparison, a control group not subjected to enucleation or intraperitoneal injections was included.

Adoptive Immunotherapy

Tumor-containing eyes were enucleated on day 40 after intracameral tumor transplantation, and adoptive transfer of TIL began on day 42. Mice were assigned randomly to one of four groups: group 1 received intraperitoneal injections of 0.5 ml complete RPMI twice daily for 5 days as a control; group 2 received intraperitoneal injections of 10,000 U IL-2 twice daily for 5 days; group 3 received 2 x 10^7 TIL administered intravenously; and group 4 received 2 x 10^7 TIL administered intraperitoneally twice daily for 5 days. For comparison, a control group not subjected to enucleation or intraperitoneal injections was included.

Isolation and Expansion of Tumor-Infiltrating Lymphocytes

Tumor-containing eyes were removed 14 days after tumor transplantation and were placed in complete Rosewell Park Memorial Institute (RPMI) medium. The anterior segment of the eye was removed and placed in a separate petri dish, and the tumor was carefully dissected from the eye under a dissecting microscope. Pooled tumors were minced with scissors, placed in 10 ml of Hank's balanced salt solution (HBSS) containing 10 mg collagenase (grade IV; Sigma, St. Louis, MO), 25 U hyaluronidase (Sigma), and 1.0 mg DNase (Sigma), and they were incubated for 1/2 hours on a rocking platform at 37°C. The cell suspension was centrifuged, then the pellet was pressed through a sterile wire mesh screen, washed twice with HBSS, resuspended in complete RPMI (see below), and passed through nylon mesh (Tetko, Elmsford, NY). Tumor-infiltrating lymphocytes were cultured in vitro with recombinant IL-2, as described.

Briefly, TIL were suspended in RPMI containing 10% heat-inactivated fetal calf serum, 1% L-glutamine, 1% sodium pyruvate, 1% antibiotic-antimycotic solution, and 50 g/ml gentamicin (= complete RPMI). Tumor-infiltrating lymphocytes were cultured at 1 x 10^7 cells/ml and cultured in 24-well culture plates in complete RPMI containing 100 U of human recombinant IL-2 per milliliter (kindly supplied by Frederick Cancer Research and Development Center, Frederick, MA). Tumor-infiltrating lymphocytes were stimulated with 2 x 10^6 x-irradiated (2,500 cGy) 124E2 tumor cells per well. After 7 days of culture with stimulator cells, the nonadherent TIL were separated from the plastic-adherent tumor cells by vigorous pipetting with RPMI medium. Tumor-infiltrating lymphocytes were transferred to 75 cm^2 culture flasks (1 x 10^7 cells/ml) and cultured for 7 more days in complete medium supplemented with fresh recombinant IL-2.

Hepatic Metastasis Model

124E2 tumor cells were implanted orthotopically into the eyes of anti-CD4-treated FVB/N and nude mice at a dose of 1 x 10^7/5 μl. Intraocular tumors grew progressively in the eyes of anti-CD4-treated FVB/N mice and athymic nude mice. Tumor-containing eyes were enucleated on day 40 after intracameral tumor transplantation, and metastatic hepatic tumor foci readily were demonstrable by histopathologic examination of livers on day 49 after intracameral tumor transplantation. 51,52

Phenotypic Analysis of Tumor-Infiltrating Lymphocytes

Tumor-infiltrating lymphocyte phenotypes were determined by fluorescence-activated cell sorter (FACS) analysis using monoclonal antibodies specific for murine T-cell surface determinants. Antibodies included anti-Thy 1.2 (New England Nuclear, Boston, MA), anti-CD4 (IgG2a monoclonal antibody secreted by rat hybridoma GK 1.5), anti-CD8 (IgG2b monoclonal antibody secreted by rat hybridoma YTS169.4), anti-CD45R (PharMingen, San Diego, CA), anti-Mac 1 (PharMingen), and anti-Asialo GM1 (Wako Chemicals, Dallas, TX). Single-cell suspensions were prepared and washed in FACS buffer consisting of phosphate-buffered saline (pH 7.4) with 1% BSA and 0.02% sodium azide. Cells (10^6) were incubated with primary antibodies for 30 minutes on ice, washed three times, and then incubated with fluorescein isothiocyanate-labeled secondary antibodies for 20 minutes at 0°C and washed three more times. Cell suspensions were fixed in 1% paraformaldehyde and assayed for positive staining on an Epics Profile Analyzer (Coulter Electronics, Hialeah, FL). Gates were set at 1% of total cells, based on staining by secondary antibodies alone.

Cytotoxic T Lymphocyte Assay

Spleen cells and TIL isolated from immunocompetent FVB/N mice in the various experimental groups were assessed for cytotoxic activity against 124E2 ocular tumor cells using a 51Cr-release assay, as described. B16 melanoma served as a target cell control to rule out nonspecific killing of neural crest-derived tumor cells by TIL effector cells. Effector spleen cells or TIL were tested either directly or after boosting in vitro. In vitro boosting was achieved by culturing 3 x 10^7 lymphoid...
cells with $6 \times 10^5$ x-irradiated (3000 cGy) 124E2 tumor cells for 4 days. Tumor cells were labeled with 0.1 mCi Na$_2$CrO$_4$ (New England Nuclear), washed three times in HBSS, and resuspended in complete medium. Stained spleen cells were placed in 96-well microtiter plates with target cells at E:T ratios ranging from 100:1, 50:1, 25:1, and 12.5:1. Four hours later, the plates were centrifuged at 1000 rpm for 5 minutes, and 0.1 ml of supernatant was collected and counted in a gamma counter. The cytotoxicity was calculated as follows:

$$\frac{\text{Experimental cpm} - \text{spontaneous release cpm}}{\text{Total release cpm} - \text{spontaneous release cpm}} \times 100.$$

Total release counts per minute were determined by treatment of target cells with 0.05 ml Zapoglobin II (Coulter Diagnostics, Hialeah, FL) and spontaneous release by incubating target cells with complete medium in the absence of effector cells.

**Statistics**

Student's t-test was used for the statistical analysis of the various experiments. Mann-Whitney test was used for comparison of multiple group survival.

**RESULTS**

**Effect of Tumor-Infiltrating Lymphocytes Against 124E2 Cells In Vitro**

To obtain preliminary evidence regarding the mechanisms of anti-tumor activity of TIL and IL-2, we examined the in vitro sensitivity of 124E2 cells to freshly isolated TIL and cultured TIL. Tumor-infiltrating lymphocytes isolated on day 14 after tumor transplantation displayed impressive, direct, dose-dependent cytolytic activity against transgenic 124E2 tumor cells but did not lyse irrelevant B16 melanoma target cells (Fig. 1). One of the critical issues in TIL therapy is the feasibility of maintaining the cytolytic activity of a patient’s TIL for subsequent infusion. Accordingly, TIL were cultured in the presence of x-irradiated autologous tumor cells and IL-2. The cytotoxic activity of cultured TIL was sustained over a 28-day period (Fig. 2). Moreover, TIL cultured for 2 weeks in the presence of IL-2 displayed higher cytolytic activity than freshly isolated TIL (Fig. 3).

**Phenotypic Characterization of Tumor-Infiltrating Lymphocytes**

The surface phenotypes of freshly isolated and cultured TIL were characterized by FACS. Freshly isolated TIL from 20 tumor-containing eyes were pooled and found to be comprised predominantly of Thy 1.2$^+$ cells (71.4%) (Fig. 4). Small numbers of macrophages (7.2%), natural killer cells (7.3%), and B cells (4.8%) were also detected in the freshly isolated TIL preparations. The infiltrating T cells were predominantly CD4$^+$ (32.2% of total TIL), although significant numbers of CD8$^+$ cells were present (18.6% of total TIL). However, after 2 weeks of in vitro culture in the presence of x-irradiated autologous tumor cells and IL-2, the ratio of CD4$^+$ to CD8$^+$ cells shifted such that the percentage of CD4$^+$ went from 32.2% to 8.8% of total TIL. This decline coincided with a comparable increase in the number of CD8$^+$ cells, which rose from 18.6% to 50.1%. Thus, the CD4/CD8 ratio changed from 1.73:1.0 to 0.17:1.0. The percentage of Mac-1$^+$ and NK$^+$ did not significantly change after in vitro culture.

**Effect of Tumor-Infiltrating Lymphocytes Therapy Against Hepatic Metastases**

The remarkable cytolytic activity of the TIL suggested that they might be effective modalities for treating metastases. In pilot studies, metastatic tumors were readily detectable in all mice examined 49 days after intracameral transplantation. Therefore, TIL were administered intravenously on day 42, and mice were necropsied on day 49, when hepatic metastases were consistently present in untreated mice. Experimental groups (5 mice/group) consisted of mice treated with either RPMI, IL-2 only, TIL, or TIL plus IL-2. Mice were killed, and livers were collected for histlogic evaluation of micrometastases on day 49. Enumeration of individual tumor nodules was not possible because of the variability in growth patterns and the tendency of the metastatic foci to grow in large, confluent areas. Therefore, all livers (five per experimen-
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FIGURE 2. Cytotoxic activity of tumor-infiltrating lymphocytes (TIL) is sustained after in vitro culture. For up to 28 days, TIL were cultured in vitro in the presence of IL-2 and x-irradiated 124E2 stimulator cells. Cytolysis was measured in a conventional 4-hour 51Cr-release assay using an effectorto-target ratio of 50:1. Each data point represents mean ± SD.

FIGURE 3. In vitro culture increases cytolytic activity of tumor-infiltrating lymphocytes (TIL), which were cultured in vitro for 14 days in the presence of IL-2 and x-irradiated 124E2 tumor cells. Fresh TIL were tested immediately after removal from tumor-bearing eyes. Effector-to-target ratio was 50:1. Bar = mean ± SD. B16F10 murine melanoma cells served as antigen-nonspecific target cells. *P = 0.026 (Student's t-test)

FIGURE 4. In vitro culture results in a shift in the surface phenotype of ocular tumor-infiltrating lymphocytes (TIL). Freshly isolated TIL and TIL were cultured for 14 days in vitro. The antigenic phenotypes of the various populations were then evaluated by fluorescence-activated cell sorter (FACS) analysis. Results are expressed as the percentage of TIL that stained positively for the respective surface antigen. Tumor cells were excluded from FACS analysis. Some Thy 1.2 TIL did not stain positively with either anti-CD4 or anti-CD8 antibodies.

2 resulted in a remarkable reduction in hepatic metastases. Hepatic metastases developed in only one animal treated with TIL alone or TIL in combination with IL-2. Moreover, the hepatic metastatic foci in the TIL-treated mouse and the mouse treated with TIL plus IL-2 were restricted to a single focus at a blood vessel margin. Tumor-infiltrating lymphocytes had a similar anti-metastatic effect in FVB/N mice that were immunosuppressed by in vivo treatment with anti-CD4 antibody (Table 1). Treatment with TIL alone completely prevented the development of metastases in anti-CD4/CD8-treated mice, whereas extensive metastases developed in all untreated and RPMI-treated mice. It should be noted that in nude mice and anti-CD4/CD8-treated FVB/N mice, the beneficial effects of TIL could have been mediated by preventing the initial establishment of hepatic metastases, inhibiting the growth of metastases that were already present when TIL therapy was initiated, or both.

The acquisition of tumor-specific cytotoxicity through the adoptive transfer of TIL was confirmed by assessing the cytotoxic T lymphocyte (CTL) responses in each treatment group. Tumor-bearing FVB/N mice were treated with either RPMI medium, IL-2, TIL, or TIL plus IL-2 on day 42. All mice were killed 4 days later, and splenic CTL activity was tested directly in a 4-hour 51Cr-release assay. Results show that immunoincompetent hosts that received TIL, either alone or in combination with IL-2, acquired tumor-specific CTL activity detectable in the spleen (Fig. 6). By contrast, significant CTL activity did not develop in animals treated with IL-2 or medium alone. Thus, recipients of TIL acquired cytolytic effector cells that persisted for at least 4 days after adoptive transfer.
FIGURE 5. Photomicrographs demonstrating the four categories of liver metastatic lesions. (A) 0 = absence of detectable tumor foci; (B) 1+ = minimal (<10% of liver involved); (C) 2+ = moderate (10% to 25% liver involved); (D) 3+ = extensive (>25% liver involved). Margins are indicated by arrows. Hematoxylin and cosin. Bar = 40 μm.

Influence of Adoptive Immunotherapy on Survival

Ultimately, the success of immunotherapy is gauged by its capacity to prolong host survival time. Therefore, additional experiments assessed the effect of TIL treatment on host survival. Accordingly, panels of anti-CD4-treated FVB/N mice and athymic nude mice were treated with a single injection of either RPMI medium, IL-2, TIL, or TIL plus IL-2 on day 42. Animals were observed daily, and percent survival was assessed for a 100-day period. As anticipated from previous results, the administration of TIL, either alone or in combination with IL-2, produced a significant prolongation of host survival times (Figs. 7A, 7B). At the end of the 100-day observation period, 60% and 70% of the TIL-treated FVB/N mice and TIL plus IL-2-treated FVB/N mice, respectively, were alive (Fig. 7B). Virtually identical results were found in parallel experiments using athymic nude mice (Fig. 7B).

DISCUSSION

Studies in murine tumor models have shown that TIL are more effective than LAK cells in mediating tumor regression. In the current study, the efficacy of TIL isolated from intraocular tumors was assessed using a hepatic metastatic model. The prevention of hepatic metastases by TIL isolated from intraocular tumors was studied using nude mice. The results of these experiments are presented in Table 1.

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<th>Treatment group</th>
<th>Severity of Metastatic Foci*</th>
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<td>Nude Mice</td>
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<tr>
<td>Nonenucleated</td>
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<td>Medium</td>
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<td>IL-2</td>
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<td>TIL</td>
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<td>TIL + IL-2</td>
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<td>FVB/N (α-CD4/CD8)</td>
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* Tumor-containing eyes were enucleated on day 40, and mice were necropsied on day 49. The incidence of metastases was based on number of liver affected by metastatic lesions. 0 = no detectable tumor foci; 1+ = minimal (<10% of liver involved); 2+ = moderate (10% to 25% liver involved); 3+ = extensive (>25% liver involved). TIL = tumor-infiltrating lymphocytes.

TABLE 1. Prevention of Hepatic Metastases by TIL Isolated From Intraocular Tumors
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In contrast to LAK cells, TIL are predominantly comprised of T lymphocytes. Culturing TIL in the presence of IL-2 and irradiated autologous tumor cells as stimulators can expand TIL more than 100-fold. Tumor-infiltrating lymphocytes expanded in vitro can produce remarkable regressions of established pulmonary and liver metastases in mouse models. Moreover, in some models, adoptively transferred TIL can survive and retain functional antitumor activity up to 119 days after intravenous injection, even in the absence of IL-2.

The potential for applying TIL therapy to uveal melanoma is worthy of serious consideration. Enucleation is commonly used in the management of uveal melanoma, and TIL have been detected in 5% to 20% of the enucleated eyes examined in several studies. T lymphocytes represent the predominant infiltrating cell in uveal melanomas, and in situ examination of the T-cell receptor (TCR) gene expression has shown restriction of TCR use of TIL in human uveal melanomas, thereby suggesting the presence of antigen-specificity in the infiltrating T cells. Functional studies have demonstrated that TIL isolated from human uveal melanomas display significant melanoma-specific cytotoxicity in vitro. The feasibility of TIL therapy in human uveal melanoma also is supported by the natural history of this neoplasm. In approximately 50% of patients treated by enucleation, metastatic disease does not develop within the first 6 months of surgery. This time lag allows sufficient time to generate adequate quantities of TIL from enucleated eyes. To date, however, no studies have examined the efficacy of TIL in preventing or treating metastases arising from intraocular melanomas. To some, TIL therapy might represent an immunologic oxymoron. If TIL are capable of lysing uveal melanoma cells in vitro, why are they unable to mediate the rejection of primary uveal melanomas? One explanation for this apparent contradiction may lie in the unique immunosuppressive characteristics of the eye. Aqueous humor is known to contain immunosuppressive cytokines, such as transforming growth factor type beta and alpha melanocyte-stimulating hormone. These cytokines, and perhaps other immunosuppressive factors in the aqueous, might impair anti-tumor effector responses within the eye. For example, highly immunogenic syngeneic murine fibro-
sarcomas induce strong systemic delayed-type hypersensitivity responses after intracameral transplantation and become infiltrated with CD4+ and CD8+ TIL, yet delayed-type hypersensitivity lesions conspicuously are absent within the intraocular tumors.27 However, the same TIL produce impressive delayed-type hypersensitivity lesions if they are removed from the eye and transplanted to extraocular sites.27 Cytotoxic T lymphocyte responses also are silenced within the intraocular milieu. For example, P815 mastocytomas transplanted into the eyes of allogeneic Balb/c mice become infiltrated with T cells, yet they grow progressively and metastasize to the lungs, liver, spleen, brain, and lymph nodes.23 Although antigen-specific CTL precursors can be isolated from the progressively growing intraocular P815 tumor allografts, they do not undergo terminal differentiation within the intraocular environment. However, once removed from the eye, the TIL become fully activated and lyse P815 tumor cells in vitro.24 Metastases arising from the same intraocular tumor allografts undergo swift CTL-mediated rejection.43 Thus, it is reasonable to suspect that CTL in other intraocular tumors, such as uveal melanomas, are inhibited by the local environment but can be activated in vitro and exert antimitastatic effects in vivo.

The results from the present study support the feasibility of TIL therapy for treating metastases arising from intraocular melanomas. Ideally, it would have been desirable to evaluate the antimitastatic effects of TIL in the original transgenic mouse model. This was not possible, however, for humane and logistical reasons. Transgenic animals invariably develop rapidly growing bilateral tumors that blind hosts early in life.23 Moreover, the rapid tumor growth leads to death 45 to 55 days after birth.23 Presumably, the previously observed absence of spontaneous metastases in tumor-bearing transgenic hosts is due to the shortness of their lives, which does not allow sufficient time for the development of metastases.25 Orthotopic transplantation of transgenic tumors to syngeneic hosts, however, allowed us to evaluate the fundamental usefulness of TIL as antimitastatic modalities. As in other models, TIL could be cultured for several weeks without loss in cytolytic activity, thereby establishing the possibility of multiple rounds of TIL therapy if necessary. Tumor-infiltrating lymphocytes were able to eradicate hepatic metastases that arose from intraocular melanomas and that developed after the tumor-containing eyes were removed. The profound reduction in micrometastases and the long-term survival of more than 60% of the treated mice provide clear evidence that TIL isolated from intraocular tumors can be highly effective antimitastatic modalities. These results, along with previous findings indicating that TIL can be isolated from human uveal melanomas, suggest that TIL therapy should be given serious consideration for use in the management of patients with uveal melanoma. However, caution should be exercised in extrapolating the present findings to human uveal melanoma because the murine transgenic tumors possess characteristics that suggest they may be RPE carcinomas and not uveal melanomas.

Key Words:
tumor-infiltrating lymphocytes, uveal melanoma, liver, metastases

References

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