Treatment of Intracranial Tumors by Systemic Transfer of Superantigen-activated Tumor-draining Lymph Node T Cells

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ABSTRACT

Adoptive transfer of tumor-sensitized T lymphocytes has demonstrated therapeutic efficacy in animal tumor models and in some patients with melanoma and renal cell cancers. In experimental settings, T lymphocytes derived from lymph nodes (LNs) draining progressively growing tumors can be activated ex vivo to generate tumor-reactive lymphocytes with therapeutic efficacy. Despite the theoretical concern regarding inaccessibility of the central nervous system to systemically transferred T cells, our recent experiments demonstrated that anti-CD3-activated tumor-draining LN cells are capable of mediating the regression of established intracerebral tumors. In this study, several staphylococcal enterotoxins (SEs), including SEA, SEC2, and SEE, and exfoliating toxin, known to be superantigens, were tested for their ability to stimulate tumor-draining LN cells to acquire antitumor reactivity for the treatment of intracerebral tumors. SEs bind to the MHC class II molecule and provide an activating signal to T cells bearing particular T-cell receptor Vβ chains. Tumor-draining LN cells activated with SEs demonstrated selective Vβ T-cell expansion. In adoptive immunotherapy of intracranial (IC) tumors, SEA- and SEC2-activated cells had the highest efficacy, whereas SEE-activated cells were not therapeutic. Despite the antigen independence of SE activation, the T cells retained immunological specificity for the tumor, which provided the initial in vivo sensitization of the LN. During the ex vivo stimulation with superantigens, both CD4+ and CD8+ T cells proliferated, and both subsets were required to mediate regression of IC tumors. In contrast to the adoptive immunotherapy of visceral tumors, the systemic administration of exogenous interleukin 2 failed to support the antitumor reactivity in mice depleted of CD4 cells, and, in fact, it inhibited the therapeutic efficacy. Furthermore, mice cured of intracerebral tumors by the adoptive transfer of T cells were resistant to an IC tumor rechallenge. However, in contrast to the immunological specificity demonstrated during the primary adoptive transfer, cured mice were able to reject challenge with several immunologically distinct fibrosarcomas but not a melanoma. These results indicate that superantigen-activated LN cells can circulate to and interact with intracerebral tumors mediating tumor regression in an immunologically specific manner. Although conditions that optimize the treatment of intracerebral tumors appear to be different from those for visceral tumors, analysis of T-cell receptor Vβ expression among cells activated with several superantigens does not reflect a preferential usage of Vβ gene segments in the immune response to autochthonous tumors.

INTRODUCTION

It is clearly evident that during progressive tumor growth, the host contains T lymphocytes sensitized to tumor antigens, but other immunological and physiological factors may have prevented an effective antitumor response from eradicating the existing tumor. However, under certain conditions, the intentional infusion of tumor-reactive T lymphocytes may result in the regression of tumors. The use of T lymphocytes for the treatment of cancers has been referred to as "adoptive immunotherapy." Several sources of tumor-sensitized lymphocytes have been identified in cancer-bearing patients, including peripheral blood (1-4), lymphoid tissue (5-7), and TILs3 (8-10). Murine models have established that an abundant source of tumor-sensitized T lymphocytes is present in LN-draining s.c. tumors (11, 12). T cells from tumor-bearing mice are largely incapable of mediating tumor regression when immediately transferred into secondary tumor-bearing hosts (11, 12). However, these cells, when properly activated ex vivo, undergo a functional change such that they mediate regression of established tumors on adoptive transfer (12).

Several ex vivo activation methods have been developed for tumor-sensitized LN cells involving the use of tumor cells as well as T-cell-activating reagents. Tumor-reactive cells can be generated using mAbs directed against the € chain of the TCR-CD3 complex (12, 13). This antigen-independent stimulation permits the activation of tumor-reactive cells in cases in which sufficient tumors are unavailable or in which the relevant tumor antigens are not characterized. Recently, we have characterized the antitumor reactivity of superantigen-activated tumor-draining LN cells (14). Bacterial superantigens are strong mitogens for T lymphocytes bearing certain TCR Vβ regions (15). Treatment of tumor-draining LN cells with SEA or SEB led to the selective activation and expansion of certain TCR Vβ subsets. The adoptive transfer of SEB-activated LN cells resulted in the immunologically specific regression of established pulmonary metastases (14). To determine whether LN cells stimulated with SEs could effectively treat tumors in an immunologically privileged site, we used a model of experimentally induced IC tumor.

The CNS has long been considered an immunologically privileged site. Several factors may contribute to this status, including an endothelial structure that restricts passage of macromolecules, viruses, and cells; the absence of lymphatic drainage; and inadequate expression of MHC molecules (16, 17). Results from immunotherapy trials for malignant melanoma by either active immunization or adoptive transfer of T cells suggest that the CNS may be relatively inaccessible to tumor-reactive cells, because some patients developed disease progression in the brain despite tumor regression at other sites (18). For this reason, patients with CNS metastases are usually excluded from immunotherapy trials. Attempts have been made to explore the potential of adoptive immunotherapy for primary CNS malignancies, because a more favorable prognosis can be predicted by increased tumoral lymphocyte infiltration (19, 20). However, results from numerous clinical trials of adoptive immunotherapy for brain tumors have thus far been disappointing (21, 22).

Despite previous difficulties in generating an adequate immune response in the CNS, there are reasons to believe that T lymphocytes, under appropriate conditions, can penetrate the blood-brain barrier and mediate therapeutic effects. In experimental models, active immunization of animals with allogeneic cells or syngeneic melanoma cells resulted in the immunologically specific rejection of challenges in the brain with the allogeneic or tumor cells (23-25). Moreover, T-cell-mediated autoimmune responses exist in naturally occurring diseases such as multiple sclerosis and in experimental allergic en...
T-cell activation can be achieved with the use of superantigen molecules. To understand the factors that improve this method of activation is not feasible. Alternatively, restricted LN cells must contain a large proportion of T cells that lack the tumor antigenic peptides presented in context with MHC molecules (31–33). In the absence of chemically and molecularly defined tumor antigens, immunologically specific T lymphocytes could be best activated and expanded via stimulation with the interactions of TCRs and tumor antigenic peptides presented in context with MHC molecules (31–33). In this study, tumor-draining LN cells activated with several staphylococcal superantigens were examined for the adoptive immunotherapy of experimentally induced IC tumors. Because of the VB specificity of the superantigen stimulation, an additional goal was to determine whether there is overexpression of the TCR VB regions in the immune response of LN to syngeneic tumors.

MATERIALS AND METHODS

Mice. Female C57BL/6J (hereafter called B6) mice were purchased from The Jackson Laboratory (Bar Harbor, ME), were at least 8 weeks of age, and weighed approximately 20 g at the time of experiments. Mice were housed in a specific pathogen-free environment and were fed food and water ad libitum.

Tumors. The 3-methylcholanthrene-induced fibrosarcomas MCA 205 and MCA 207 originally derived in B6 mice were kindly provided by Dr. James C. Yang (National Cancer Institute, NIH, Bethesda, MD; Ref. 34). These tumors have been maintained in vivo by serial s.c. transplantation in syngeneic mice and were used at the third to eighth transplantation passages. Single-tumor cell suspensions were prepared by digesting minced tumor in 40 ml HESS (Life Technologies, Grand Island, NY) containing 4 mg DNase I, 40 mg collagenase type IV, and 100 units hyaluronidase type V (Sigma Chemical Co., St. Louis, MO) for 3 h at room temperature. The tumor cells were filtered through a layer of Nytex 100 nylon mesh (Tetko, Inc., Briarcliff Manor, NY), washed three times with HBSS, and resuspended at the appropriate concentrations for IC or s.c. inoculations. They were harvested, washed once, and resuspended in CM with 2 X 10@/ml in gas-permeable culture bags (Baxter/Fenwall, Deerfield, IL). After three additional days of incubation, the cells were harvested and resuspended in HBSS for adoptive immunotherapy.

Adoptive Immunotherapy. Three days after IC inoculation with tumor cells, mice received sublethal WBI, 500 cGy delivered from a 137Cs irradiator source (Mark I irradiator; J. C. Shepard & Associates, Glendale, CA) before adoptive cell transfer. Previous experiments demonstrated that WBI augmented the efficacy of the adoptive transfer of IC tumors, although it had minimal effects on tumor growth in the absence of cell transfer (30). Mice received 1 X 106 and 3 X 106 ex vivo-activated effector cells or 1.2 X 108 freshly isolated tumor-draining LN cells in 1 ml through the tail vein. In some experiments, mice were also treated with IL-2 (10,000 units i.p. twice daily for eight doses). Mice were followed for survival or were euthanized by CO2 inhalation when death appeared imminent. The criteria used to determine imminent death were palpable bulging of the skull and decreased spontaneous and induced motor activity. These criteria were exhibited by animals within 1–2 days of spontaneous death.

SEs. The enterotoxin SEA was purchased from Sigma. SEC2, SEE, and ExFt were obtained from Toxin Technology, Inc. (Sarasota, FL).

Fluorescence-activated Cell Sorting Analysis. Cells (5 X 106) were incubated with appropriately diluted primary antibodies. FITC-conjugated mouse mAb to rat @ chain (MRK-1; Pharmingen, San Diego, CA), goat antimouse immunoglobulin (Kirkegaard & Perry Laboratories, Gaithersburg, MD), or goat antimouse immunoglobulin (Cappel; Organon Teknika Corp., Durham NC) was used depending on the species of the primary antibody. Stained cells (106) were analyzed on a FACScan (Becton Dickinson, Sunnyvale, CA). The percentage of positive cells was calculated by subtracting the background staining of the negative control and normalized based on the percentage of CD3+ cells.

Tumor Inoculation. Mice were anesthetized with 0.6–0.8 mg pentobarbital i.p. Inoculation of 4 X 106 or 8 X 106 tumor cells (10 ml tumor cell suspension of 4 or 8 X 106 cells/ml) was performed transcranially over approximately 20 s, using a 27-gauge stainless steel needle and glass tuberculin syringe (Perfectum; Popper & Sons, Inc., New Hyde Park, NY). The needle insertion was perpendicular to the skull and in line with the anterior margin of the ear and the medial half of the right eye. The depth of insertion was controlled by placement of electric wire insulation as a collar over the needle with exposure of the terminal 4 mm of the needle.

Activation of Tumor-draining LNs. s.c. tumors were established in B6 mice by inoculation with 1.5 X 106 tumor cells in 0.05 ml HBSS in the flank region bilaterally. Twelve days later, inguinal LNs draining the tumors were resected under sterile conditions, teased apart with 20-gauge needles, and pressed with the blunt end of the plunger of a 10-ml plastic syringe. The cell suspension from the combined LNs was filtered through a No. 100 nylon mesh, washed, and resuspended in CM (RPMI 1640 supplemented with 10% heat-inactivated FCS, 0.1 mm nonessential amino acids, 1 mm sodium pyruvate, 2 mm glutamine, 100 mg/ml streptomycin, 100 units/ml penicillin, 50 mg/ml gentamicin, 0.5 mg/ml amphotericin B, all from Life Technologies), and 5 X 105 ml 2-mercaptoethanol (Sigma)).

LN cells suspended at 4 X 106/ml were stimulated by SEs at the following concentrations: SEA, 20 ng/ml; SEC2, 1 mg/ml; SEE, 1 mg/ml; and ExFt, 1 mg/ml. Cells were cultured at 1 ml/well in 24-well plates in 5% CO2 at 37°C for 2 days.

Anti-CD3 was immobilized onto 24-well tissue culture plates, which had been precoated overnight at 4°C with staphylococcus-coccus A (Sigma) (0.3 ml of a 100 mg/ml solution in PBS/well). Anti-CD3 (0.3 ml of a 2 mg/ml mAb suspension in PBS) was incubated at 4°C for 4 h. Wells were washed twice with PBS prior to the addition of 2 ml LN cells at a concentration of 2 X 106 cells/ml. Cells were cultured for 2 days in 5% CO2 at 37°C.

Cells activated with either SEs or anti-CD3 were harvested, washed once, and resuspended in CM with 4 Cetus units/ml IL-2 (kindly supplied by Chiron Therapeutics, Emeryville, CA; 1 Cetus unit = 6 IU) at a concentration of 2 X 106/ml in gas-permeable culture bags (Baxter/Fenwall, Deerfield, IL). After three additional days of incubation, the cells were harvested and resuspended in HBSS for adoptive immunotherapy.

4 Unpublished observations.
T-Cell Subset Depletion. Treatment of mice to deplete either the CD4 or CD8 T-cell subset was performed immediately after adoptive transfer of effector cells. Either rat IgG, anti-CD4 mAb (L3T4, GK1.5), or anti-CD8 mAb (Lyt2, 2.43), was administered by i.v. injection of 0.2 ml mAb ascites diluted to 1.0 ml with HBSS. On imminent death of mice, spleens from two representative animals in each group were harvested, and flow cytometry was performed to confirm the success of T-cell subset depletion.

IC Tumor Rechallenge. Mice surviving for >60 days after adoptive transfer of activated tumor-draining LN cells were rechallenged in the contralateral cerebral hemisphere with MCA 205 (4 × 10⁵ cells), MCA 207 (4 × 10⁵), MCA 203 (4 × 10⁴), or a clone of Bl6/BL6 melanoma (D5) cells (2 × 10⁴). Normal mice similarly inoculated served as controls.

Statistical Analysis. Analysis of survival was performed by the Mann-Whitney rank sum test.

RESULTS

Proliferation and Phenotype of SE-activated Tumor-draining LN Cells. Single-cell suspensions were prepared from LN-draining s.c. MCA 205 tumors for 12 days. The LN cells were activated with either immobilized anti-CD3 mAb or one of the SEs: SEA, SEC2, SEE, or ExFT. During the 2-day SE incubation, the total number of cells did not increase; however, the percentage of T cells increased from approximately 30 to 90%. The activated cells were washed and resuspended in CM with IL-2 (4 units/ml) for an additional 3 days prior to analysis or adoptive transfer. This sequence of culture induces proliferation of cells but maintains their immunological specificity (14).

As shown in Table 1, there was adequate proliferation in all of the cultures, ranging from 1.6-fold expansion in the culture activated with ExFT to 3.2-fold expansion in anti-CD3-activated cultures. Nearly all of the cells were T lymphocytes at the end of the culture period, and because the initial LN cell preparation contains approximately 35% T lymphocytes, the expansion of T cells ranged from 4.7- to 9-fold. SEs bind to MHC class II molecules and interact with particular Vβ regions of the TCR. This Vβ specificity is the most notable phenotypic difference between cells activated with different SEs, whereas anti-CD3 activated T cells with a broad range of Vβ types. The list of Vβ subtypes tested for in Table 1 is not exhaustive but confirms the Vβ restriction of superantigen activation and is in accordance with previously published studies (36).

Therapeutic Efficacy of SE-activated LN Cells against IC Tumors. Mice bearing 3-day MCA 205 tumors were treated with sublethal WBI (500 cGy) prior to adoptive cell transfer. In an initial experiment, 1.2 × 10⁶ freshly isolated tumor-draining LN cells were administered i.v. Compared with mice that received no cell transfer, the median survival was not statistically different (17 versus 16 days; P > 0.05). This result, in which fresh tumor-draining LN cells were not effective against IC tumors, is consistent with our previous experience in a lung metastases model (11, 12). Tumor-draining LN cells were activated with SEs to determine whether they acquired therapeutic efficacy for IC tumors following ex vivo stimulation. As demonstrated in Fig. 1, adoptive transfer of 1.5 × 10⁷ LN cells activated with SEA and SEC2 were highly effective, whereas anti CD3 and ExFT were less effective against IC MCA 205 (P < 0.05 versus controls), and SEE-activated cells were ineffective. In addition, SEA-, SEC2-, and anti-CD3-activated cells were superior to SEE-activated cells (P < 0.05). An additional identically designed experiment confirmed the relative therapeutic efficacy of various SE-activated cells (data not shown). Interestingly, the survival time of mice that were not cured by ExFT or anti-CD3 treatment was similar to that of mice failing treatment with SEE.

Although there were some differences in ratios of CD4⁺:CD8⁺ cells in various SE-activated cells (Table 1), this parameter did not correlate with therapeutic efficacy. For example, the CD4:CD8 ratio of the SEA-activated culture was 1.95, compared with 0.56 for the SEC2-activated cultures. Therapeutic efficacy was also not correlated with the presence or absence of a particular Vβ subset. SEC2-activated cultures were highly restricted to the Vβ 8.2 subset, which was minimally present in SEA-activated cultures. On the other hand, Vβ3 and Vβ11 subsets were amplified in cultures stimulated with SEA, which were therapeutic, and in SEE-activated cultures, which were not therapeutic.

The Role of CD4 and CD8 T Cells in Adoptive Immunotherapy and the Effects of Systemic Exogenous IL-2. Both CD4⁺ and CD8⁺ T-cell subsets were needed for the treatment of IC tumors. Depletion of either cell population in the SEC2-activated cell popu-
Fig. 3. Inhibitory effect of IL-2 on the treatment of IC tumors by the adoptive transfer of activated tumor-draining LN cells. Mice (n = 5) were treated on day three (D3) after IC tumor inoculation by the adoptive transfer of 3 x 10^6 LN cells activated with SEC2. A, IL-2 (10^6 units) or HBSS was administered i.p. twice daily for 4 days starting immediately after cell transfer. B, IL-2 treatment was started at the indicated times after tumor inoculation and continued for 4 days.

The inhibitory effect of IL-2 could be demonstrated by delaying the initiation of IL-2 treatment for up to 3 days after adoptive transfer (Fig. 3B). Systemic IL-2 in the absence of cell transfer did not change the median survival time of mice with IC tumors.

The activation protocol for these experiments includes culture in low-dose IL-2 following superantigen activation. During the 3-day culture in IL-2, T cells usually expand 6–10-fold in number. The relative therapeutic activity of LN cells after 2-day SEC2 activation or after an additional 3 days of culture in IL-2 was compared by adoptive transfer into mice bearing 3-day IC tumors. As shown in Fig. 4, all mice were cured by transfer of the highest cell dose studied, 9 x 10^6 cells, activated with SEC2 alone or after an additional 3-day culture in low-dose IL-2. Interestingly, at a dose of 3 x 10^6 cells, at which intermediate efficacy was observed, there was not a statistically significant difference in the tumor regression rate (P > 0.05). This indicates that during expansion of T cells in IL-2, the antitumor reactivity per cell did not increase or decrease significantly.

The Antitumor Response Is Initially Tumor Specific but Displays Cross-Reactivity on Tumor Rechallenge. The activation of T cells by SEs occurs indirectly through the presentation of SE molecules by MHC class II molecules and certain Vβ3 elements of the TCR. Because this interaction is independent of the antigen-specific T-cell recognition, the immunological specificity of the antitumor response was tested. In a crisscross experiment, cells derived from LN-draining MCA 205 tumors and activated by SEC2 were found to be effective against IC MCA 205 tumors but not against the antigenically distinct MCA 207 tumor (P = 0.0079; Fig. 5). Similarly, SEC2-activated MCA 207 tumor-draining LN cells were only active against IC MCA 205 but not MCA 205 tumors (P = 0.009). This result demonstrates, as have previous experiments, that the antitumor response mediated by the activated LN cells is highly specific, and the specificity is determined during the in vivo sensitization of tumor-draining LNs.

Animals cured of MCA 205 IC tumors for more than 60 days were rechallenged with a second IC tumor inoculation. These animals rejected the second inoculum of the same tumor (Fig. 6). Unexpectedly, mice cured of IC MCA 205 tumors often rejected secondary challenges with antigenically distinct but histologically related tumors, including other weakly immunogenic fibrosarcomas, MCA 207, and MCA 203. The survival of mice cured of IC MCA 205 tumors challenged with MCA 207 or MCA 203 tumors was not statistically different than that of mice rechallenged with MCA 205 tumors (P = 0.1049 and 1, respectively). However, this cross-reactivity did not extend to another tumor of a different histological type. The D5 tumor, a subclone of the B16/BL6 melanoma, grew progressively, with only a slight delay in most MCA 205-cured recipients in comparison to naive mice. There was also a statistically significant difference in survival for MCA 205-cured mice rechallenged with MCA 205 compared with D5 (P = 0.0019). However, even for this histologically unrelated tumor, several animals demonstrated prolonged survival.

DISCUSSION

Tumor-draining LNs contain a mixture of cells, including MHC class II-positive, antigen-presenting cells such as macrophages, dendritic cells, and B cells that are capable of presenting SEs for T-cell stimulation. During the first 2 days of superantigen stimulation, the total cell number did not increase; however, the percentage of T cells increased from approximately 35% to greater than 90%, indicating

Fig. 4. Comparison of the antitumor activity of tumor-draining LN cells activated for 2 days with SEC2 or after expansion in IL-2-containing medium for an additional 3 days. Graded doses of activated cells were infused i.v. into mice (n = 5) bearing 3-day IC tumors.

Two Day Activation

Five Day Activation

4705

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IMMUNOTHERAPY OF BRAIN TUMORS WITH SAg-ACTIVATED T CELLS

Fig. 5. Specificity of the antitumor response mediated by the adoptive transfer of activated tumor-draining LN cells. Mice (n = 5) bearing either IC MCA 205 or MCA 207 tumors were treated by the adoptive transfer of 3 × 10^7 SEC2-activated cells derived from LNs draining either MCA 205 or MCA 207 tumors.

selective T-cell proliferation. Vigorous T-cell proliferation became clearly evident during the ensuing 3-day culture with IL-2. The total expansion of T lymphocytes ranged from 5- to 9-fold over the 5-day culture period, and virtually all of the recovered cells were T lymphocytes. Because the Vβ subsets stimulated by the SEs were initially a minority of the T-cell population, their relative expansion was even greater than 5–9-fold. One of the critical requirements for the development of clinical adoptive immunotherapy is the ability to rapidly expand populations of T cells in vitro. The results from this study suggest that superantigen stimulation may afford a means to generate a large number of T cells for clinical use. However, because T-cell stimulation with superantigens requires the presentation by MHC class II-positive accessory cells, additional stimulation of activated cells may require the presence of these cells. In humans, activated T lymphocytes often express MHC class II molecules on their surface. Whether these induced molecules are sufficient to present superantigens remains to be confirmed.

The impetus for the current study using superantigen-stimulated cells for immunotherapy came from numerous documented observations of restricted use of TCR gene segments that were selectively expressed on T cells infiltrating solid tumors (40–43). Most of these analyses were carried out in patients without the simultaneous consideration of HLA alleles. Recently, a number of genes encoding melanoma antigens, recognized as peptides presented by MHC class I molecules to CTLs, have been identified (44–50). In a patient population matched for HLA-A2, TCR Vβ14 was demonstrated to be

Fig. 6. Cross-reactive antitumor response in mice cured of IC tumors by the adoptive transfer of activated LN cells. Mice cured of IC MCA 205 tumors by the adoptive transfer of 3 × 10^7 SEC2-activated LN cells were rechallenged more than 60 days after the initial tumor inoculation. The fibrosarcomas MCA 207, MCA 203, and melanoma D5 were inoculated in the contralateral hemisphere from the original MCA 205 tumor (n = 8/group), and survival time after the secondary tumor challenge was determined. Naive mice (n = 5) were simultaneously inoculated with an identical dose of the indicated tumors.
overexpressed at the site of primary melanoma lesions (51). However, a lack of preferential TCR gene segment use has also been reported in malignant melanoma (52).

If there is selection in TCR use in response to malignant tumor cells, it should be readily demonstrated with defined transplantable tumors in syngeneic animals. Such studies have been reported by several groups of investigators. For example, the immune response to the MOPC-3.5 plasmacytoma appeared to be restricted to the CD8+ TCR Vβ8.3 CTLs (53). A preferential use of the TCR Vβ10 gene was reported to be the predominant T-cell immune response in spontaneous regression of the virally induced FBL-3 leukemia (54). In contrast, analysis of distribution of Vβ usage by TILs from progressively growing methylcholanthrene-induced fibrosarcomas and a dimethylhydrazine-induced colon adenocarcinoma of B6 origin revealed a broad distribution of Vβ phenotypes; although differences in frequency in comparison to spleen cells were observed (55). When TILs were examined for Vβ use after a period of in vitro culture, individual cultures rapidly became dominant for a single Vβ phenotype. There was, however, no association of a specific Vβ with tumor specificity. Although these data suggest the complexity of immune responses to syngeneic tumors, it is also clear that different T-cell phenotypes can independently mediate antitumor effects.

Our results are consistent with the conclusion that the selection of tumor-specific T cells is not reflected in any simple predominance of Vβ use. The panel of SAs we tested induced the activation and proliferation of several Vβ T cells, including Vβ3, Vβ5, Vβ8.2, and Vβ11. Antitumor efficacy did not seem to correspond to the prevalence of a particular Vβ subset or its absence. For example, two Vβ species, Vβ3 and Vβ11, were amplified in cultures stimulated with SEA, which are therapeutically effective, as well as in cultures stimulated with SEE, which were ineffective. To further support this conclusion, our recent study with the MCA 106 fibrosarcoma indicates that at a clonal level, T cells with several Vβ phenotypes can equally mediate tumor regression (28).

Treatment of T lymphocytes with antibodies against CD3 or exposure to SEs has been reported to cause apoptosis in vivo and in vitro (56–58). Under the activation conditions used in these experiments, we observed continual proliferation through the duration of culture and functional antitumor activity on adoptive transfer. One possibility is that the T cells sensitized in vivo to tumor antigens were at a stage of differentiation that permitted proliferation and activation by SE exposure. However, the equivalent activity of cells after only 2-day SEC2 activation and cells after subsequent expansion in IL-2 for an additional 3 days does not support this conclusion. Because 3-day incubation with IL-2 resulted in an increase in net effector cell numbers, we routinely include IL-2 expansion as part of the ex vivo activation procedure. The presence of antigen-presenting cells in the lymph node culture during activation may have influenced proliferation of SE-stimulated cells, as has been observed in other systems (59, 60). The antitumor immune response persists in the host and mediates resistance to rechallenge.

Analogous to the adoptive immunotherapy of the same tumor, MCA 205, at a different histological site (e.g., lung), an effective antitumor response to IC tumors required both CD4+ and CD8+ subsets. In a separate study, we have demonstrated by immunohistochemistry the presence of SEC2-activated Vβ8.2+ T cells of both CD4+ and CD8+ subsets infiltrating IC tumors following i.v. adoptive transfer of SEC2-activated LN cells (61). In previous experiments in which pulmonary MCA 205 metastases were treated with anti-CD3- or SEB-activated T cells, the administration of systemic IL-2 (15,000 units twice daily for eight doses) restored therapeutic efficacy in CD4+ cell-depleted animals, and in general, systemic IL-2 enhanced the function of mixed cultures consisting of both CD4+ and CD8+ cells (11, 14). In contrast, for the treatment of IC tumors, IL-2 inhibited the antitumor efficacy of transferred cells. This inhibition of effector cell function is apparent even if the IL-2 dose is delayed for 48 h after cell transfer. The mechanism of inhibition by IL-2 has not yet been determined. Perhaps IL-2 affects the ability of effector cells to migrate to the IC tumor site. IL-2 can cross the blood-brain barrier and induces vascular leak at the site of CNS tumors (62, 63). It may thus impose inhibitory effects in the local environment of the CNS.

The observation of considerable cross-reactivity between several antigenically distinct tumors on rechallenge of cured animals was interesting but puzzling in light of the exquisite immunological specificity displayed by the transferred cells during the primary rejection response. Cross-reactivity was not observed for all tumors tested; in fact, following inoculation of the B16/B16 melanoma tumor line D5, there was only a slight prolongation in survival for most MCA 205-cured mice compared with naive mice. It is possible that additional immune responses were generated during tumor rejection against epitopes that are shared by other closely related malignant tumor lines, in particular, fibrosarcomas. T cells with a phenotype that facilitates their migration across the CNS vasculature may have been selectively amplified during the primary rejection response. Alternatively, the primary antitumor response may have induced long-term changes in either the vasculature or the activity of nonspecific effector cells within the CNS, which may facilitate the rejection of tumors with shared or closely related antigenic epitopes. Mice cured of IC tumors displayed normal behavior during a prolonged observation period after tumor regression, suggesting that any of the possible changes in the immunologically privileged status of the CNS did not significantly affect neurological function. Because the tumor cell lines used in these studies are not derived from CNS tissue, the question of whether immune responses to cross-reactive neural epitopes develop in the regression of primary CNS neoplasms remains. Future studies with tumors derived from glial or neural elements will help address these questions and further define the criteria for successful adoptive immunotherapy of IC tumors.

REFERENCES


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