Realization of the Therapeutic Potential of CTLA-4 Blockade in Low-Dose Chemotherapy-treated Tumor-bearing Mice

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Abstract

CTLA-4 blockade has been shown by other investigators [D. R. Leach, et al., Science (Washington DC), 271: 1734–1736, 1996; and Y.-F. Yang, et al., Cancer Res., 57: 4036–4041, 1997] to retard tumor growth in selected tumor systems. Here, we show that CTLA-4 blockade alone was ineffective in retarding tumor growth in the murine MOPC-315 tumor system. Yet, CTLA-4 blockade offered significant therapeutic benefits to MOPC-315 tumor bearers when combined with a subtherapeutic dose of the chemotherapeutic agent melphalan, which was previously shown (L. Gorelik, et al., Cancer Immunol. Immunother., 39: 117–126, 1994) to shift the cytokine profile in the tumor bearers toward type-1 cytokines. In addition, we show here that anti-CTLA-4 monoclonal antibody enhanced antitumor cytokoticity when the anti-CTLA-4 monoclonal antibody was added to stimulation cultures of spleen cells from low-dose melphalan-treated MOPC-315 tumor-bearing mice but not from untreated tumor-bearing mice. These results suggest that the therapeutic benefits of CTLA-4 blockade depend on the ability of drugs such as melphalan to promote an immunogenic environment by altering the cytokine profile of tumor-specific T cells.

Introduction

It has been recognized for some time that progression of many murine and human tumors is due to the inability of the tumors to elicit effective tumor-eradicating immunity. This in turn was attributed to multiple factors that included low immunogenicity of the tumor cells as a result of the absence or low-level expression of tumor Ag/MHC-complex (1) or the lack of expression of the costimulatory molecules B7-1 and/or B7-2 (2). In fact, many attempts to enhance the ability of tumor cells to elicit the generation of tumor-eradicating immunity focused on increasing the ability of the tumor cells to provide both signal 1 and signal 2 for T-cell activation (e.g., by introducing genes for MHC class I molecules (3) or for the costimulatory molecule B7-1 or B7-2 (2, 4)). However, these manipulations provided at best some therapeutic benefits against a relatively small (barely palpable) tumor burden (4). The failure of such manipulations to provide therapeutic benefits to mice with larger tumors may have been due at least in part to the production of tumor-associated cytokines with inhibitory activity for the generation of cell-mediated antitumor immunity (5–7). In contrast to the therapeutic benefits provided by CTLA-4 blockade in the V51BLim10, the CSA1M fibrosarcoma and the OV-HM ovarian carcinoma.

In contrast to the therapeutic benefits provided by CTLA-4 blockade in the V51BLim10, the CSA1M, and the OV-HM tumor systems, CTLA-4 blockade alone does not always provide therapeutic benefits as reported in mid August 1998 by Hurwitz et al. (15) in the Sm1 mammary carcinoma system. Similarly, we show herein that CTLA-4 blockade alone also does not provide therapeutic benefits in the MOPC-315 tumor system. However, the therapeutic potential of CTLA-4 blockade can be realized in the MOPC-315 tumor system after administration of low-dose melphalan under conditions in which the chemotherapy promotes the in vivo acquisition of CD8+ T-cell-mediated tumor-eradicating immunity (16). Consistent with the possibility that the therapeutic benefits of CTLA-4 blockade for low-dose melphalan-treated tumor-bearing mice are due at least in part to the ability of anti-CTLA-4 mAb to lead to enhanced antitumor immunity, the addition of anti-CTLA-4 mAb to stimulation cultures of spleen cells from low-dose melphalan-treated MOPC-315 tumor-bearing mice, but not to spleen cells from untreated tumor-bearing mice, was found to result in enhanced anti-MOPC-315 cytotoxicity.

Materials and Methods

Tumors. The MOPC-315 plasmacytoma was maintained in vivo in female BALB/c AnNcrBR mice 7–10 weeks old (Charles Rivers Breeding Laboratories, Wilmington, MA). Unless otherwise stated, mice were inoculated s.c. with 1 × 106 viable tumor cells, a dose that is at least 300-fold higher than the minimal lethal tumor dose.

Chemotherapy. A fresh stock solution of melphalan (Sigma Chemical Co., St. Louis, MO) was prepared as described previously (17). A dose of 1.5–2.0 mg melphalan/kg body weight (low-dose) was given i.p. to BALB/c mice bearing large (~20 mm) tumors that resulted from the s.c. inoculations of 1 × 106 MOPC-315 tumor cells 10 days earlier.

Antibody Treatments. For CTLA-4 blockade, mice received a daily i.p. injection of 100 μg (per mouse) of the affinity-purified, hamster IgG anti-CTLA-4 mAb (UC10–4F10–11; Ref. 10) for up to 10 injections or until the tumor nodules reached 25 mm in diameter. This mAb was produced in our laboratory and purified by Genetic Institute (10). As a control, mice received affinity-purified Normal IgG (Sigma Chemical Co.).

Spleen CellSuspensions. Spleens used for the preparation of single-cell suspensions were derived from two sources: (a) mice bearing a large (20–22-
ever, the pattern of results remained consistent. The level of antitumor cytotoxicity 10-12 days earlier: and (ft) mice that were treated with low-dose melphalan 4 days earlier when the mice bore an ~20-mm s.c. tumor that resulted from the inoculation of 1 × 10^6 MOPC-315 tumor cells 10-12 days earlier.

**In Vitro Generation of Anti-MOPC-315 Cytotoxicity.** Spleen cells were stimulated in vitro with mitomycin C-treated MOPC-315 tumor cells according to the method we have described previously (5. 17) for the in vitro generation of CTL activity by CD8+ spleen cells from untreated or low-dose melphalan-treated MOPC-315 tumor bearers. Briefly, spleen cells were cultured at 37°C for 5 days with mitomycin C-treated MOPC-315 tumor cells in DMEM supplemented with 5% fetal bovine serum, 5 × 10^{-5} M 2-mercaptoethanol (Sigma Chemicals), 1% nonessential amino acids, 50 units/ml penicillin, 50 μg/ml streptomycin, and 15 mM HEPES buffer (Life Technologies, Gaithersburg, MD).

**Antitumor Cytotoxicity Assay.** The level of antitumor cytotoxicity exhibited by in vitro stimulated spleen cells was determined by the 51Cr release assay. Briefly, 1 × 10^6 51Cr-labeled MOPC-315 tumor cells were incubated with effector cells at three different E:T ratios. The percentage of specific 51Cr release was calculated by the following formula:

\[ \frac{E^	ext{spm} - S^	ext{spm}/M^	ext{rpm} - S^	ext{spm} \times 100}{5} \]

where \( E^	ext{spm} \) represents the 51Cr released by target cells incubated with effector cells, \( S^	ext{spm} \) represents the spontaneous release and \( M^	ext{rpm} \) represents the maximal release obtained by the addition of 2% NP40 detergent (Particle Data Corp., Elmhurst, IL) solution. Some variations were noted in the levels of antitumor cytotoxicity between different experiments, however, the pattern of results remained consistent. The level of antitumor cytotoxicity of a representative experiment is presented as the mean percentage of 51Cr release of triplicate samples ± SE. In addition, to illustrate the reproducibility of our observations, the data from all of the experiments addressing the same question were converted to LU/1 × 10^7 effector cells and are presented as LU ± SE.

**Statistical Analysis.** The significance of differences in the fraction of mice surviving after different treatments was determined by the generalized Savage (Mantel-Cox) test. For all of the other statistical analyses, Student’s t test was used. A P value of ≤ 0.05 was considered significant in both tests.

**Results and Discussion.**

In light of reports that anti-CTLA-4 mAb treatment of mice bearing a V51BLimir10 (13), CSA1M (14), or OV-HM (14) tumor leads to a substantial reduction in tumor growth and even regression of a few of the tumors, experiments were carried out to determine whether anti-CTLA-4 mAb treatment would offer some therapeutic benefits also to mice bearing the MOPC-315 plasmacytoma. As seen in Fig. 1, anti-CTLA-4 mAb treatment initiated on day 7 after MOPC-315 tumor inoculation, when the mice bore an ~10 mm s.c. tumor, did not retard MOPC-315 tumor growth (Fig. 1A). Because the tumor burden at the time of initiation of the anti-CTLA-4 mAb treatment was larger in the MOPC-315 tumor system than in the V51BLimir10, CSA1M, or OV-HM tumor systems (13, 14) and because, in the V51BLimir10 tumor system, anti-CTLA-4 mAb treatment initiated at the time of tumor inoculation was also effective in inhibiting tumor growth and even preventing tumor establishment (13), we examined whether anti-CTLA-4 mAb treatment initiated at the time of MOPC-315 tumor inoculation could offer any “therapeutic” benefits. In this study, mice were inoculated s.c. with either 1 × 10^6 or 2 × 10^6 MOPC-315 tumor cells. As seen in Fig. 1, anti-CTLA-4 treatment did not inhibit significantly tumor growth when administered to mice inoculated with either 1 × 10^6 (Fig 1B) or 2 × 10^6 (Fig. 1C) MOPC-315 tumor cells. Thus, in contrast to the therapeutic benefits offered by CTLA-4 blockade in the V51BLimir10, CSA1M, and OV-HM tumor systems, CTLA-4 blockade alone did not offer any therapeutic benefits in the MOPC-315 tumor system.

**Days Post-Tumor Inoculation**

Next, we considered the possibility that the failure of anti-CTLA-4 mAb to provide any therapeutic benefits to MOPC-315 tumor bearers was due to the fact that tumor progression in the MOPC-315 tumor system (5), as in many other murine and human tumors (6, 7, 9), activates not only T cells that produce type-1 cytokines and promote the development of cell-mediated immunity but also T cells that produce type-2 cytokines, and have the potential to inhibit the generation of cell-mediated immunity. Consequently, CTLA-4 blockade would be expected to promote the activity of both kinds of activated T cells as we (18, 19) and others (20) have shown—in vivo in nontumor systems—that CTLA-4 blockade can sustain not only the production of type-1 cytokines but also the production of type-2 cytokines. Consistent with such a scenario, anti-CTLA-4 mAb treatment was found in the studies by Yang et al. (14) to offer therapeutic benefits to mice at early stages of CSA1M or OV-HM tumor growth, and spleen cells from mice bearing small tumors were found to produce elevated levels of interleukin 2 and IFN-γ in vitro upon culture with anti-CTLA-4 mAb. At the same time, anti-CTLA-4 mAb treatment did not offer any therapeutic benefits to mice that bore tumors larger than 5 mm in diameter, and spleen cells from mice bearing larger tumors did not produce elevated levels of interleukin 2 and IFN-γ in vitro upon culture with anti-CTLA-4 mAb.

As a first step toward the testing of our hypothesis, we carried out studies to determine whether the therapeutic potential of CTLA-4 blockade can be realized in the MOPC-315 tumor system when the balance is shifted through external manipulations toward T cells that are involved in the generation/exertion of tumor-eradicating immunity. For this purpose, we took advantage of our previous observations, which demonstrated that the administration of low-dose melphalan to mice bearing large (~20 mm in diameter) MOPC-315 tumors shifts the cytokine profile in favor of type-1 cytokines (5, 8) and leads to the development of potent CD8+ T-cell-dependent antitumor immunity that in turn eradicates a large tumor mass (16, 17).

In the current studies, we used a suboptimal dose of melphalan (1.5...
In summary, the results presented herein illustrate that in a tumor system in which CTLA-4 blockade alone is unable to offer any therapeutic benefits, the therapeutic benefits of CTLA-4 blockade can still be realized through the use of melphalan. In addition, our data suggest that melphalan allows for the realization of the therapeutic benefits of CTLA-4 blockade in the MOPC-315 tumor system, at least in part, by allowing for the realization of the ability of CTLA-4 blockade to lead to enhanced antitumor immunity. Thus, CTLA-4 signaling is one of multiple immunosuppressive activities in a tumor-bearing host, and future immunotherapies should be targeted not only at CTLA-4 blockade but also at the removal/neutralization of other factors with inhibitory/down-regulatory activity for effective antitumor immune responses.

References


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