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 tumor cytotoxicity 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 fact, recent attempts to enhance the ability of tumor bearers to mount tumor-eradicating immunity have focused on the shift in the cytokine profile of the tumor site toward cytokines with stimulatory activity for the generation of cell-mediated immunity through the use of chemotherapeutic agents such as melphalan (5, 8), cyclophosphamide (7), or bleomycin (9).

Another factor described recently (10–14) that may limit the effectiveness of tumor-eradicating immunity is CTLA-4/B7 interaction because CTLA-4, which is expressed on activated T cells (10), functions as a negative regulator of T-cell responses. In fact, CTLA-4 blockade (through the use of anti-CTLA-4 mAb) was reported by Leach et al. (13) to lead to the inhibition of tumor growth as well as the complete regression of a few palpable tumors in the V51BLim10 colon carcinoma model. These observations by Leach et al. were recently extended by Yang et al. (14) to two other experimental tumor models, 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 NcNcrBR 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 IgG (Sigma Chemical Co.).

Spleen Cell Suspensions. Spleens used for the preparation of single-cell suspensions were derived from two sources: (a) mice bearing a large (20–22-
mm) s.c. tumor that resulted from the inoculation of 1 × 10⁶ MOPC-315 tumor cells 10–12 days earlier; and (b) 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 × 10⁵ 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 × 10⁶ 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:

\[
E_{	ext{pm}} - S_{	ext{pm}}/M_{	ext{pm}} - S_{	ext{pm}} \times 100
\]

where \( E_{	ext{pm}} \) represents the ⁵¹Cr released by target cells incubated with effector cells, \( S_{	ext{pm}} \) represents the spontaneous release and \( M_{	ext{pm}} \) 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 × 10⁷ 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 V51BLim10 (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 V51BLim10, CSA1M, or OV-HM tumor systems (13, 14) and because, in the V51BLim10 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 × 10⁶ or 2 × 10⁶ 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 × 10⁶ (Fig 1B) or 2 × 10⁶ (Fig. 1C) MOPC-315 tumor cells. Thus, in contrast to the therapeutic benefits offered by CTLA-4 blockade in the V51BLim10, CSA1M, and OV-HM tumor systems, CTLA-4 blockade alone did not offer any therapeutic benefits in the MOPC-315 tumor system.
factors with inhibitory/down-regulatory activity for effective antitu-
mat CTLa-4 blockade but also at the removal/neutralization of other
benefits of CTLa-4 blockade in the MOPC-315 tumor system, at least
therapeutic benefits, the therapeutic benefits of CTLa-4 blockade can
cytotoxicity.

suggest that melphalan allows for the realization of the therapeutic
benefits of CTLa-4 blockade alone is unable to offer any

of mice surviving out of the total mice studied; *, statistically significant extension in
survival time relative to that of mice treated with melphalan plus NlgG.

mg/kg), which is curative for only 25–50% of the MOPC-315 tumor
bearers because of the appearance of insufficient CD8⁺ T-cell-mediated
antitumor immunity (8, 16), and determined whether anti-
CTLA-4 treatment would offer any therapeutic benefits to these mice. As seen in Fig. 2, treatment of MOPC-315 tumor bearers with anti-
CTLA-4 mAb improved significantly the curative effectiveness of the
suboptimal dose of melphalan, with ~70% of the mice alive and
tumor-free at the end of a 70-day observation period, as compared with only ~40% of the mice in the melphalan-plus-NlgG treatment
group.

Experiments were next carried out to determine whether
CTLA-4 blockade could lead to enhanced anti-MOPC-315 cyto-
taxicity when added to stimulation cultures of spleen cells from
low-dose-melphalan-treated MOPC-315 tumor bearers but not
when added to stimulation cultures of spleen cells from untreated
tumor-bearing mice. Specifically, spleen cells from untreated
MOPC-315 tumor-bearing mice or from low-dose-melphalan-
treated tumor-bearing mice were stimulated in vitro for 5 days with
MOPC-315 tumor cells in the presence or absence of anti-CTLA-4
mAb, and subsequently the spleen cells were evaluated for their
antitumor cytotoxicity by the 51Cr release assay. As seen in Fig. 3,
the addition of anti-CTLA-4 mAb to stimulation cultures of spleen
cells from untreated tumor-bearing mice did not lead to an en-
hanced (but actually led to a somewhat suppressed) anti-MOPC-
315 cytotoxicity. In contrast, the addition of anti-CTLA-4 mAb to
stimulation cultures of spleen cells from low-dose-melphalan-
treated MOPC-315 tumor bearers led to enhanced anti-MOPC-315
cytotoxicity.

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 antitu-
mor immune responses.

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Fig. 2. The effect of anti-CTLA-4 mAb on the curative effectiveness of low-dose
melphalan for mice bearing a large s.c. MOPC-315 tumor. Mice bearing a large (~20
mm in diameter) day-10 s.c. tumor were treated with 1.5 mg/kg melphalan plus 100 μg of
anti-CTLA-4 (●) or NlgG (□) daily beginning 2 h after the chemotherapy. As a reference
point, we provide information regarding the survival time of mice treated with NlgG (●)
or anti-CTLA-4 mAb (□) but without chemotherapy. All of the mice that were alive on
day 70 after low-dose chemotherapy were tumor-free. Numbers in parentheses, number of
mice surviving out of the total mice studied; *, statistically significant extension in
survival time relative to that of mice treated with melphalan plus NlgG.

Fig. 3. The effect of CTLA-4 blockade on the level of anti-MOPC-315 cytotoxicity
exhibited by in vitro stimulated spleen cells from untreated or low-dose-melphalan-treated
MOPC-315 tumor bearers. Spleen cells derived from untreated mice bearing a large (~20
mm s.c. tumor (top panels) or mice that were treated with low-dose (2.0 mg/kg)
melphalan when they bore an ~20-mm s.c. MOPC-315 tumor (bottom panels) were
stimulated in vitro with mitomycin C-treated MOPC-315 tumor cells in the presence of 50
μg/ml anti-CTLA-4 mAb (●) or NlgG (□). Five days after the initiation of the stimulation
cultures, the spleen cells were assessed for their anti-MOPC-315 cytotoxicity by the 3.5-h
51Cr release assay. The results of a representative experiment out of a total of 10
experiments are presented in the A panels as % of 51Cr release. As a reference point, we
provide information regarding the level of anti-MOPC-315 cytotoxicity exhibited by
spleen cells cultured in vitro in the absence of added stimulator tumor cells (□). The
cumulative data from all of the experiments were converted to LU and are presented in the
B panels. *, a significantly higher level of antitumor cytotoxicity than the level exhibited
by spleen cells stimulated in the presence of NlgG. The level of antitumor cytotoxicity
exhibited by tumor-bearer spleen cells that were stimulated in vitro in the presence of
anti-CTLA-4 mAb was not significantly different (P = 0.14) from the level exhibited by
tumor-bearer spleen cells stimulated in the presence of NlgG.


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