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 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 and/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 at 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 Hurwit 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 chemotherapeutic 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-bearers 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 AnN/CrlBR 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 NlgG (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-
Cytotoxicity of a representative experiment is presented as the mean per
ever, the pattern of results remained consistent. The level of antitumor
cells 10-12 days earlier: and (ft) mice that were treated with low-dose mel-
and are presented as LU ±SE.

The reproducibility of our observations, the data from all of the experiments
percentage of "Cr release of triplicate samples ± SE. In addition, to illustrate
the levels of antitumor cytotoxicity between different experiments, how
the maximal release obtained by the addition of 2% NP40 detergent

Antitumor Cytotoxicity Assay. The level of antitumor cytotoxicity exhibited
by in vitro stimulated spleen cells was determined by the "Cr release
assay. Briefly, I X 10^4 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:

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

where \(E_{pm}\) represents the 51Cr released by target cells incubated with
effector cells, \(S_{pm}\) represents the spontaneous release and \(M_{pm}\) 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, how-
ever, the pattern of results remained consistent. The level of antitumor
cytotoxicity of a representative experiment is presented as the mean per-
centage 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 X 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 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. 1A, 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 X 10^6 or 2 X 10^6 MOPC-315 tumor
cells. As seen in Fig. 1, anti-CTLA-4 treatment did not inhibit sig-
nificantly tumor growth when administered to mice inoculated with
either 1 X 10^6 (Fig 1B) or 2 X 10^6 (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.

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 immu-
nity. For this purpose, we took advantage of our previous observa-
tions, which demonstrated that the administration of low-dose mel-
phalan 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 anti-
tumor immunity that in turn eradicates a large tumor mass (16, 17).
In the current studies, we used a suboptimal dose of melphalan (1.5
mor immune responses. Signaling is one of multiple immunosuppressive activities in a tumor-bearing mouse, and subsequently the spleen cells were evaluated for their antitumor cytotoxicity by the 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 survival time of mice treated with NlgG (•) or anti-CTLA-4 mAb (○) 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.

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 cytotoxicity 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 enhanced (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 antitumor immune responses.

References


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