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Provision of Granulocyte-Macrophage Colony-Stimulating Factor Converts an Autoimmune Response to a Self-Antigen into an Antitumor Response

Qingyong Ji,*† David Gondek, † and Arthur A. Hurwitz†*†

Many tumor Ags recognized by T cells are self-Ags. Because high avidity, self-reactive T cells are deleted in the thymus, any residual self-reactive T cells existing in the periphery are likely to be low avidity and nonresponsive due to peripheral tolerance mechanisms. Activation of these residual T cells is critical for targeting tumors for immunotherapy. In this study, we studied immune responses against the murine B16 melanoma using a tyrosinase-related protein 2 (TRP-2) peptide as a model tumor/self-Ag. Our results showed that TRP-2 peptide vaccination alone elicited a weak T cell response and modestly decreased B16 lung tumor nodules. The combination of peptide vaccination and treatment with an Ab directed against the inhibitory receptor CTLA-4 enhanced the immune response against TRP-2 peptide, inducing autoimmune depigmentation and further decreasing lung tumor nodules. However, both vaccination methods failed to protect against orthotopic (s.c.) B16 tumor challenge. The addition of an irradiated GM-CSF-expressing, amelanotic tumor cell vaccine significantly delayed s.c. B16 tumor growth. Subsequent studies revealed that provision of GM-CSF increased dendritic cell numbers in lymph nodes and spleen. Furthermore, addition of CTLA-4 blockade increased the frequency of TRP-2-specific, IFN-secreting T cells in spleen and lymph nodes. Overall, our results indicate that combining enhancement of Ag presentation with removal of CTLA-4-mediated inhibition can convert a “weaker” autoimmune response into a more potent antitumor immune response.

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*Abbreviations used in this paper: TRP-1, tyrosinase-related protein 1; TRP-2180–188, aa 180–188 from TRP-2; DC, dendritic cell; LN, lymph node; MHC-I, MHC class I.

Tumor cells can be targeted and destroyed by T cells through TCR recognition of a short antigenic peptide/MHC complex on the surface of tumor cells. Ag receptor occupancy alone is not sufficient because priming of naive T cells also requires a costimulatory signal mediated in part by CD28 ligation by its ligands, CD80 or CD86 (collectively referred to as B7), which are expressed by APCs. Interestingly, the majority of tumor Ags recognized by T cells are actually “self” Ags that are tissue-specific differentiation Ags; gp100, melanoma antigen recognized by T-cells 1 (MART-1), tyrosinase, tyrosinase-related protein 1 (TRP-1)2, and TRP-2 are melanoma Ags also expressed by melanocytes that provide potential T cell epitopes (1). Because most self-reactive, high avidity T cells are eliminated in the thymus during development, the majority of self-reactive T cells that persist in the periphery are low avidity and remain nonresponsive to self-Ags through a variety of potential mechanisms: anergy, ignorance, or suppression. Thus, overcoming this nonresponsiveness remains a critical obstacle in generating a potent antitumor immune response (2–4).

Once activated, T cells express CTLA-4, an inhibitory receptor that also binds B7 ligands (5, 6). Because of its higher avidity for B7, CTLA-4 efficiently competes for ligand binding with CD28.

Engagement of CTLA-4 by B7 decreases IL-2 secretion, arrests cell cycle progression, and down-regulates T cell activation and proliferation (7, 8). Several studies have demonstrated that specifically blocking the interaction of CTLA-4 with B7 enhanced immune responses in vitro and in vivo (9–11).

Using both transplantable and primary murine tumor models, we and others (12–14) demonstrated that blockade of CTLA-4 with an anti-CTLA-4 Ab can enhance antitumor immunity, although the efficacy correlated with the tumor’s inherent immunogenicity. Treatment with anti-CTLA-4 Ab promoted regression of several immunogenic, transplantable murine tumors, but not poorly immunogenic tumors, like the B16 melanoma or SM1 mammary carcinoma. Our subsequent studies demonstrated that these tumors were also rejected when CTLA-4 blockade was combined with other therapies (15–17). In one of these studies, we reported that recently established melanoma melanoma B16-BL6 tumors were cured by the combination of a GM-CSF-expressing cell vaccine and CTLA-4 blockade (17). More profound was the observation that these B16-immune mice developed hair depigmentation, indicating that the immune response was, at least in part, directed against Ags expressed by melanocytes. Further investigation showed that T cells from cured mice recognized aa 180–188 from TRP-2 (TRP-2180–188), an enzyme involved in melanin synthesis [dopachrome tautomerase, DCT (18)].

Based on the above results, we hypothesized that CTLA-4 blockade may help break tolerance to self-Ags and enhance the autoimmune response to prevent tumor growth. To test this hypothesis, we studied autoimmunity and tumor immunity following vaccination with the TRP-2180–188 peptide. Our findings demonstrated that mice sensitized to TRP-2 in combination with CTLA-4 blockade developed autoimmune depigmentation. However, immunity to s.c. B16 tumors was dependent on the additional provision of GM-CSF, which resulted in increased accumulation of dendritic cells (DC). These findings suggest that whereas lowering the threshold for T cell activation may facilitate autoimmune
reactivity, immunity against a poorly immunogenic tumor requires recruitment of APCs.

**Materials and Methods**

**Mice**

Six- to 8-wk-old C57BL/6 female mice were purchased from the National Cancer Institute Animal Production Area Facility (Frederick, MD), maintained in a specific pathogen-free facility, and used for experiments at the ages of 7–9 wk. All vaccinations and tumor challenge were performed after mice were anesthetized with isoflurane (Midas). Mice were treated in accordance with the National Institutes of Health Guidelines under protocols approved by the Institutional Animal Care and Use Committees.

**Cell lines and Abs**

Murine melanoma cell line B16-BL6 and B16-F10 and the thymoma cell line EL4 were cultured in DMEM supplemented with 10% heat-inactivated FCS, 2 µM l-glutamine, 1 U/ml penicillin, and 1 µg/ml streptomycin at 37°C in a humidified incubator with 5% CO2. The GM-CSF-expressing mammary carcinoma cell line GMSM1 was prepared as described previously (15) and was grown in Eagle’s MEM with Earle’s balanced salt solution medium containing 10% heat-inactivated FCS, 2 µM l-glutamine, 1 mM sodium pyruvate, 1x nonessential amino acid, 1 U/ml penicillin, and 1 µg/ml streptomycin at 37°C in a humidified incubator with 5% CO2. When used as a vaccine, GMSM1 cells were lifted from dishes with trypsin/EDTA solution (BioWhittaker), irradiated using 10,000 rad, and washed three times with HBSS (BioWhittaker). GM-CSF concentration was ~300 ng/10⁶ cells/24 h.

Hamster anti-mouse CTLA-4 mAb (clone 9H10) was generated from supernatant or ascites and purified at the lab, as described previously (15). G1K1.5 (anti-CD4) and 2.43 (anti-CD8) mAbs were made as ascites through standard protocols. Hamster IgG and rat IgG were purchased from Jackson ImmunoResearch Laboratories.

**Peptide**

TRP-2180–188 (SVYDFFVWL) peptide was purchased from New England Peptide, and the purity was >90% based on HPLC assay. Peptide was dissolved in DMSO and diluted with water or complete medium and used for vaccination and in vitro studies.

**Vaccination and depigmentation observation**

TRP-2180–188 was emulsified with equal volume of CFA (Difco) or IFA (Difco). Two hundred microliters (150 µg of peptide) of the emulsions were injected s.c. into two sites on the shaved dorsal area of mice. One hundred micrograms anti-CTLA-4 Ab or control Ab hamster Ig was given i.p. on days 2, 5, and 8 after vaccination. Mice were then challenged with B16 tumor 14 days later or were monitored for depigmentation over the subsequent 2 mo. Depigmentation was graded as follows: 0, no depigmentation; 1, random small patch of white hairs; 2, patch size <0.5 cm; 3, patch size between 1 and 2 cm; 4, patch size >2.0 cm.

**Tumor challenge**

B16-BL6 or B16-F10 tumor cells used for tumor challenge were cultured at <50% confluence and were less than five passages after thawing from liquid nitrogen storage. For s.c. tumor challenge, 2 wk after vaccination, mice received injections s.c. with 1 × 10⁶ tumor cells in 100 µl of HBSS (BioWhittaker). Tumor size, determined by the multiplicative product of perpendicular diameters, was monitored with a caliper every other day. When the tumor size exceeded 250 mm², mice were euthanized, and anti-CTLA-4 mAb 9H10 or control Ab hamster Ig was given i.p. three times at 3-day intervals, starting on the same day as vaccination. Pulmonary tumor nodules were counted between 20 and 25 days after tumor challenge.

**Lymphocyte subset depletion**

Lymphocyte subsets were depleted by injecting Abs GK1.5 (anti-CD4), 2.43 (anti-CD8), or a combination of the two mAbs at days −8, −7, and −6 relative to Ag challenge, and every 5 days thereafter until the end of experiments. Depletion was confirmed by testing lymph nodes (LN) and spleens 1 day before vaccination using noncross-reactive Abs. Routinely, <1% CD4⁺ T cells, or CD8⁺ T cells were detected in LN and spleen, but mice treated with control rat IgG demonstrated unchanged lymphocyte population.

**Cytotoxicity assays and IFN-γ secretion assay**

Two to 3 wk after vaccination, spleens were removed and were pressed between two sterile slides to prepare a single-cell suspension. After lysing of RBC, the splenocytes were washed twice and cultured (1 × 10⁵ cells/well) with 0.2 µM TRP-2180–188 peptide in 12-well plates at 37°C in 3 ml of complete DMEM. After 5 days, these splenocytes were harvested and used as effector cells for cytotoxicity assay with the JAM test or IFN-γ ELISA.

For the JAM test, EL-4 cells were labeled with 5 µCi/ml tritiated thymidine overnight at 37°C. Half of the labeled cells were then pulsed with 0.4 µM TRP-2180–188 peptide for another 2 h. After thorough washing, 5 × 10⁴ EL-4 cells or TRP-2-pulsed EL-4 cells were then cocultured in V-shaped 96-well microtiter plates with effector cells at indicated E:T ratios (triplicate for each ratio). After 4 h of incubation at 37°C, cells were then harvested onto fiberglass filter mats, and radioactivity was determined by liquid scintillation counter. The percentage lysis was calculated using the following formula: ((S − E)/S) × 100, where cpm in the absence of effector cells was defined as S and that in the presence of effector cells was defined as E.

For IFN-γ secretion assay, 2.5 × 10⁵ effector cells were cocultured with 1 × 10⁵ stimulator cells in round-bottom 96-well microtiter plate for 24 h. The peptide concentration was the same as that for the JAM test. Supernatant was then collected and tested for the presence of IFN-γ by sandwich ELISA.

**Determination of DC number**

Seven days after vaccination, pooled draining LN (inguinal, axillary, and brachial) and spleen were cut into small fragments and digested with 1 mg/ml collagenase IV (Sigma-Aldrich) and 0.2 µg/ml DNase (Ambion) in 1 ml of complete medium with intermittent agitation for 30 min at 37°C. A total of 0.5 µM EDTA was then added and cultured for another 5 min to disrupt DC-T cell complexes. The suspension was then filtered through a 70-µm cell strainer to remove aggregates and stromal material. After total cell number was counted, 1 × 10⁶ cells were stained with FITC-conjugated anti-CD11c (clone N418; eBioscience) and PE-conjugated anti-I-A/I-E (clone M5/114,15.2; BD Biosciences) or isotype control FITC-conjugated Armenian hamster IgG (clone 8C1; BD Biosciences) or PE-conjugated rat IgG (clone R3-34; BD Biosciences) for 30 min on ice. The stained cells were analyzed on a BD FACScan flow cytometer (BD Biosciences). DC number was calculated by the product of percentage of CD11c and I-A/E double-positive cells, percentage of gated cells, and total cells from each LN or spleen.

**ELISPOT assay**

Mice were euthanized, and cells from spleen or draining LN (inguinal, axillary, and brachial) were prepared into a single-cell suspension. Multiscreen filtration plates (Millipore) were coated with 10 µg/ml anti-mouse IFN-γ (clone R4-6A2; BD Biosciences) in PBS (50 µl/well) overnight at 4°C. The plates were washed three times with PBS and blocked with complete medium for 2 h at 37°C. A total of 5 × 10⁵ splenocytes or LN cells and different concentrations of TRP-2180–188 were added to a final volume of 100 µl/well (triplicate per concentration). Plates were incubated for 36 h at 37°C. After washing three times with PBS and three times with PBS washing buffer (PBS and 0.05% Tween 20), plates were incubated with 100 µl of 5 µg/ml biotinylated rat anti-mouse IFN-γ (clone XMG1.2; BD Biosciences) in PBS/0.5% BSA for 2 h at 37°C. Plates were washed six times with PBS washing buffer. Fifty microliters of streptavidin-conjugated alkaline phosphatase (Rockland; diluted 1/2000 in PBS) were added to each well. Plates were incubated at room temperature for 45 min. Plates were rinsed three times with PBS washing buffer and three times with PBS, and IFN-γ spots were developed with 50 µl of 5-bromo-4-chloro-3-indolyl phosphate/NBT phosphate substrate (Kirkegaard & Perry Laboratories) for 5 min at dark. Plates were then rinsed with water to stop the reaction and air-dried. IFN-γ-specific spots were counted with the ImmunoSpot Analyzer (Cellular Technology, with the assistance of the Laboratory of Cell Mediated Immunity, National Cancer Institute, Frederick, MD).
Results
Blockade of CTLA-4 during vaccination enhances Ag-specific immune response and causes depigmentation

TRP-2 (dopachrome tautomerase) is a critical enzyme for pigment synthesis in melanocytes (19). We previously reported that mice sensitized to a GM-CSF-expressing B16 vaccine and treated with anti-CTLA-4 developed an anti-TRP-2-180-188 T cell response. In our initial experiments, we tested whether sensitizing mice to TRP-2-180-188 peptide in combination with CTLA-4 blockade could activate TRP-2-180-188-specific T cells. Two weeks after priming, splenocytes were briefly restimulated in vitro, and cytotoxic activity was evaluated. As shown in Fig. 1A, TRP-2 peptide vaccination alone induced a weak but detectable response against TRP-2-180-188-pulsed target cells. In contrast, blockade of CTLA-4 at the time of sensitization significantly enhanced the cytotoxicity against TRP-2-180-188 (Fig. 1B). The response was specific because splenocytes from sensitized mice killed TRP-2-pulsed, but not unpulsed, target cells. Splenocytes from naive mice did not show any cytotoxicity against TRP-2-pulsed target cells (data not shown). Similarly, splenocytes from TRP-2-180-188-vaccinated/anti-CTLA-4-treated mice secreted IFN-γ when cocultured with TRP-2-180-188-pulsed EL-4 cells or B16-F10 melanoma cells (Fig. 1C). We observed minimal cytotoxicity directed against B16 cells, although some enhancement was observed when B16 cells were pretreated with IFN-γ.

The autoimmune consequence of sensitization to a pigmentation Ag on melanocytes was investigated by observing the loss of hair color, a feature of vitiligo-like depigmentation. Mice were vaccinated s.c. with TRP-2 peptide in adjuvant as described previously and treated systemically with either a control or an anti-CTLA-4 Ab and monitored for depigmentation. We consistently observed that TRP-2 peptide vaccination and treatment with the control Ab did not cause depigmentation (data not shown). In contrast, peptide vaccination in combination with CTLA-4 blockade elicited depigmentation in >60% of mice (18 of 28) within 2 mo postvaccination, with onset beginning as early as 30 days postsensitization (Fig. 2A). The depigmentation was smaller compared with that of mice vaccinated with GM-CSF-expressing B16 cells and presented as a small patch of white hair adjacent to the vaccination site. Thus, the above results indicate that CTLA-4 blockade can enhance T cell responses against self-Ags and promote autoimmune depigmentation.

TRP-2-180-188 vaccination and CTLA-4 blockade fail to protect against s.c. B16 tumor challenge

We next sought to determine whether the autoimmune depigmentation was associated with an antitumor immune response as well. Mice were challenged s.c. with 1 × 106 B16-BL6 tumor cells 2 wk postvaccination, and tumor growth was monitored. As shown in Fig. 3, all mice vaccinated with TRP-2 peptide, irrespective of Ab treatment, developed tumor. Tumor growth after ant-CTLA-4 treatment was not significantly different from that of naive mice or control Ab-treated mice (p > 0.05). In many instances, although mice developed depigmentation after blockade of CTLA-4, tumor immunity was not elicited (Fig. 2B).

Lack of tumor immunity was not subline specific because similar results were obtained following challenge with another B16 subline, B16-F10 (data not shown). Boosting vaccination, increasing Ag dose, or up-regulation of MHC class I (MHC-I) on tumor cells before implantation did not alter tumor growth either (data not shown). Similarly, this sensitization approach was not effective at treating recently established B16 tumors either (data not shown). Thus, vaccination with a single tumor epitope, in combination with
CTLA-4 blockade, did not protect against orthotopic B16 tumor challenge, despite induction of autoimmune depigmentation.

**CTLA-4 blockade decreases lung metastases in both the prophylactic and therapeutic setting**

Pulmonary metastases are a common site of secondary disease among melanoma patients. When injected i.v., B16 tumor cells traffic to the lung and can serve as a model for therapy of melanoma metastasis. We tested whether peptide vaccination and anti-CTLA-4 mAb injection could protect against pulmonary metastasis formation. Two weeks after vaccination, mice were challenged i.v. with $6 \times 10^5$ B16-BL6 cells. Fourteen days later, mice were euthanized, and lung surface tumor nodules were enumerated. As shown in Fig. 4A, when compared with naive mice, vaccination with TRP-2 peptide and injection of control Ab significantly inhibited tumor growth in the lungs ($p < 0.05$). CTLA-4 blockade further decreased the number of tumor nodules ($p < 0.05$ compared with control Ab injection). Protection of lung metastasis tumor was also tested with B16-F10 subline as well. The results were similar to that of B16-BL6 challenge in that CTLA-4 blockade significantly decreased the accumulation of lung tumor nodules (Fig. 4B; $p < 0.05$ compared with control Ab).

Based on the results from lung metastasis protection experiment, we predicted that TRP-2_180–188 vaccination and CTLA-4 blockade may have therapeutic effects for lung metastases as well. One day after i.v. B16-BL6 tumor challenge, mice were vaccinated with TRP-2_180–188 and treated with anti-CTLA-4 Ab. As shown in Fig. 4C, TRP-2 vaccination and injection of control Ab decreased the tumor load in the lungs ($p < 0.05$ compared with naive mice), whereas blockade of CTLA-4 decreased the tumor nodule counts further ($p = 0.05$ compared with control Ab injection). In contrast, no therapeutic effect of TRP-2 sensitization was observed when mice were vaccinated 3 days after tumor challenge (data not shown). Thus, immunity to systemic tumor challenge is restricted by the time elapsed after tumor challenge, which presumably is a function of the size of the pulmonary nodules.

Depigmentation and lung tumor protection after CTLA-4 blockade is mediated by CD8 T cells

The TRP-2_180–188 epitope used to sensitize mice in this study is H-2Kb-restricted. To confirm that the immune responses were CD8$^+$ T cell-mediated, T cell depletion studies were performed. CD4$^+$, CD8$^+$, or both CD4$^+$ and CD8$^+$ T cells were depleted before sensitization, and mice were observed for depigmentation or challenged i.v. with B16-BL6 tumor cells. As shown in Fig. 5A, no depigmentation was noted in mice depleted of CD8$^+$ T cells or mice depleted of both CD4$^+$ and CD8$^+$ T cells. In contrast, depigmentation was unaffected in CD4 T cell-depleted mice or control-depleted mice. In some mice, depigmentation was more profound in mice depleted of CD4$^+$ T cells alone (Fig. 2C).

A similar effect of lymphocyte depletion was observed for protection from B16 pulmonary nodules (Fig. 5B). That is, depleting CD8$^+$ T cells and double depletion of both CD4$^+$ and CD8$^+$ T cells abolished protection from systemic tumor challenge. Thus, not surprisingly, immune responses against TRP-2_180–188 are dependent on CD8$^+$ T cells.

**Combination of GM-CSF and CTLA-4 blockade delays s.c. B16 tumor growth**

Taken together, our previous findings suggest that enhancement of Ag presentation is critical for eliciting potent tumor immunity (15,
GM-CSF and CTLA-4 Blockade Protect against Tumor Challenge

We next addressed the mechanism by which immunity was enhanced following provision of GM-CSF. Because GM-CSF is a cytokine with that affects the growth and differentiation of DCs (20), we studied the density and phenotype of DCs in the vaccine-draining LN and the spleen. Mice were vaccinated with the GMSM1 vaccine and treated with anti-CTLA-4 Ab. Six days later, the draining LN and spleen were removed, and DC phenotype and frequency was assessed by flow cytometric analysis.

TRP-2 vaccination alone increased DC number in draining LN (p = 0.01 compared with naive mice; Fig. 7A). However, provision of GM-CSF significantly increased DC density even further (p < 0.001 compared with TRP-2 vaccination alone). The DC number in the LN of mice vaccinated with TRP-2 alone was 5-fold greater than the number in naive mice and 10-fold greater in GM-CSF-treated mice. DC density was the highest in mice treated with both TRP-2 vaccination and GM-CSF (p < 0.001). Comparable increases in DC number were also observed in the spleen (data not shown). Unexpectedly, GM-CSF did not profoundly increase the expression of MHC or costimulatory molecules. No changes in CD86 and CD40 expression were noted, and only an incremental change in CD80 expression was noted (Fig. 7B).

We hypothesized that the increase in the density of DCs following provision of GM-CSF may, in turn, result in a greater expansion of TRP-2-specific T cells. Therefore, we also tested the effect of providing GM-CSF on the frequency of TRP-2-specific T cells in draining LN (Fig. 8A) and spleen (Fig. 8B) using an IFN-γ ELISPOT assay. In draining LN, the frequency of TRP-2-reactive T cells was greater in mice sensitized to TRP-2 in combination with GM-CSF and anti-CTLA-4 (p = 0.001). In those mice, we observed up to a 4-fold greater frequency of TRP-2-reactive T cells compared with singly treated mice (Fig. 8B).

GM-CSF injection and CTLA-4 blockade increase the frequency of TRP-2-specific T cells and DCs

Discussion

In this study, we demonstrate that sensitization to a pigmentation Ag in combination with CTLA-4 blockade enhanced the immune responses against a self-Ag, resulting in the development of autoimmune depigmentation and protection against the formation of lung tumor nodules. However, immunity to s.c. tumor challenge required the added provision of GM-CSF at the time of priming. The addition of GM-CSF resulted in an increased frequency of melanoma Ag-specific T cells and an increase in DC density on the draining LN.
Due to thymic deletion of autoreactive T cells, the peripheral repertoire of T cells recognizing self-Ags is believed to be restricted and composed primarily of low avidity T cells. Activation of these cells is considered an obstacle in generating a potent antitumor immune response. Previous studies have demonstrated that vaccination of mice with DNA vaccines encoding human or mouse melanocyte Ags such as gp100, TRP-2, and TRP-1 could break tolerance and induced tumor immunity and/or autoimmunity (21–24). Still other approaches have used peptide-pulsed DCs to induce tumor immunity against self-Ags with concomitant tumor immunity (25, 26). Previously, we demonstrated that vaccination with a B16 cell-based vaccine, in combination with CTLA-4 blockade can induce immunity to B16 associated with autoimmunity and TRP-2180–188 (17, 18). In this study, we show that CTLA-4 blockade can help break tolerance to a single peptide of TRP-2 and enhance the immune responses. However, using this approach, tumor immunity was restricted. The mechanism by which CTLA-4 blockade enhances tumor immunity is not yet clear. It is possible that CTLA-4 blockade helps to prime these self-reactive T cells by lowering the threshold of signals needed for their activation. In this scenario, T cells that express low avidity Ag receptors may become activated when CTLA-4-mediated inhibitory signals are removed. This is in contrast to the findings of Egen and Allison (31) who suggested that CTLA-4 may be preferentially expressed following higher-affinity TCR interactions. Further studies to characterize the avidity of anti-TRP-2 T cells will help resolve this disparity.

Breaking tolerance to melanoma Ags can cause an autoimmune response that manifests as depigmentation and tumor rejection (21–23). Following sensitization to a minimal epitope, we observed that autoimmunity depigmentation was accompanied by immunity to pulmonary metastases but not s.c. tumors. Failure to reject s.c. tumor challenge was unexpected and was not due to the loss of Ag expression, a common way for tumors to escape T cells (32, 33). Given that TRP-2-specific CTLs cause depigmentation, the failure to protect against s.c. tumor challenge is not likely due to the inability of TRP-2-specific T cells to traffic to the s.c. site, but rather may be due to accessibility to the tumor bed itself. The influence of organ environment on the immunological destruction of tumors has been reported by others. Krauss et al. (34) reported that adoptive transfer of ex vivo-cultured T cells could demonstrate that tolerance to tumor-associated self-Ags can be broken following CTLA-4 blockade, but tumor immunity is restricted.

FIGURE 5. Autoimmune depigmentation and lung tumor protection requires CD8+ T cells. C57BL/6 mice were depleted CD4, CD8 T cells, or both cell populations with corresponding Abs 6 days before vaccination. Mice were vaccinated as described in Fig. 1 and then observed for depigmentation for 2 mo (A) or challenged i.v. with 6 × 10^6 B16-BL6 tumors (B). Depigmentation data were combined from two separate experiments. Tumor challenge data were representative of two separate experiments with 7–8 mice per group.

FIGURE 6. Combining GM-CSF and CTLA-4 blockade delays s.c. melanoma BL6 growth. C57BL/6 mice were sensitized s.c. with TRP-2180–188 on day 0, and 5 × 10^5 irradiated GMSM1 cells were injected near the vaccination sites on day 0 and repeated on day 3. One hundred micrograms anti-CTLA-4 mAb or control hamster Ig was injected i.p. on day 2, 5, and 8. A total of 10^4 B16-BL6 cells was implanted s.c. on day 14. Tumor size was measured every other day and is presented as tumor size (A, C) or tumor incidence (B, tumor >9 mm²). B, p < 0.05 (log rank test); C, p < 0.01 (unpaired t test). The results are representative of two separate experiments with 7–8 mice per group.
treat established pulmonary tumors but had no effect on s.c. tumor. Further studies from the same group demonstrated that successful treatment of s.c. tumor required transfer of a larger number of T cells and participation of both CD4\(^+\) and CD8\(^+\) T cells (35). Our finding that the provision of GM-CSF results in a greater expansion of TRP-2-specific T cells and concomitant tumor immunity supports these latter findings and underscore the importance of increasing Ag presentation and the consequent magnitude of the immune response in determining therapeutic efficacy.

Another possible explanation for the disparity in tumor immunity may be the relative accessibility of the immune system to the site of tumor growth. In the lung tissues, which are highly vascularized and hemopoietic in nature, activated T cells may have greater access to the tumor lesions. In contrast, s.c. tumors tend to be more heavily encapsulated and potentially less-accessible. The fact that both the Bl6 and the F10 sublines have similar susceptibilities in the lung and s.c. tissues supports the idea that locale of tumor growth strongly influences the susceptibility to immunotherapy.

Our data also demonstrate that provision of GM-CSF was sufficient to convert a weaker autoimmune response into a more potent antitumor immune response that was capable of slowing the growth of s.c. B16 tumors. Provision of GM-CSF increased DC density in the vaccine-draining LN and also increased the frequency of TRP-2-specific T cells. Somewhat surprisingly, there was no detectable change in DC phenotype following provision of GM-CSF. This suggests that the magnitude of the APC response is critical for eliciting a sufficiently potent antitumor response and that use of a simple adjuvant does not provide adequate recruitment of APCs. Our findings do not exclude the possibility that using a GM-CSF-expressing amelanotic vaccine (like SM1) may also provide a “helper” effect, although the SM1 vaccine alone did not enhance B16 immunity. Ongoing studies will help clarify this issue.

Our findings presented here imply that blocking CTLA-4 may simply lower a threshold for T cell priming and result in activation of a defined number of self-reactive T cells that is insufficient to provide protection to poorly accessible s.c. tumors. Alternatively, CTLA-4 blockade may prolong T cell activation events (e.g., TCR engagement or costimulatory interactions) and sustain or augment ongoing T cell responses. However, the synergy of CTLA-4 blockade with GM-CSF-induced enhancement of Ag presentation to TRP-2-specific T cells resulted in T cell expansion to a level capable of inducing immunity to s.c. B16 tumors. Therefore, these data have important implications for the design and modification of clinical trials testing these therapeutic regimens.
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Disclosures
A. A. Hurwitz is a coauthor on a patent involving CTLA-4/GM-CSF.

References

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