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CTL-Dependent and -Independent Antitumor Immunity Is Determined by the Tumor Not the Vaccine[1]

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Previously, we compared the efficiency of direct injection with an adenovirus (Ad) expressing human gp100 (hgp100) to immunization with dendritic cells (DC) loaded with the same vector ex vivo. The DC vaccine provided the greatest protection against challenge with B16F10 melanoma, and antitumor immunity was found to be $CD8^+$ T cell-independent. In the current study, we sought to determine whether lack of $CD8^+$ T cell-mediated antitumor immunity was a function of the vaccine platform or the tumor line. Both Ad and DC/Ad vaccines elicited $CD8^+$ CTL reactive against hgp100 and provided protection against B16F10 engineered to express hgp100 demonstrating that both vaccination platforms can effectively generate protective $CD8^+$ T cell-mediated immunity. The hgp100-induced CTL cross-reacted with murine gp100 (mgp100) and lysed B16F10 cells pulsed with mgp100 peptide indicating that the resistance of B16F10 cells to CTL elicited by hgp100 vaccination may be due to a defect in processing of the endogenous mgp100. Indeed, introduction of the TAP-1 cDNA into B16F10 rendered the cells sensitive to lysis by gp100-specific CTL. Furthermore, gp100-immunized mice were protected from challenge with B16F10-TAP1 cells through a mechanism dependent upon $CD8^+$ T cells. These results demonstrate that tumor phenotype, not the vaccination platform, ultimately determines $CD8^+$ or $CD4^+$ T cell-mediated tumor clearance. ***The Journal of Immunology*****, 2004, 172: 5200–5205.**

Studies of tumor immunology have established a correlation between the development of CTL and tumor rejection. Optimization of antitumor $CD8^+$ T cell responses has, therefore, been a major focus of cancer vaccine design. A variety of vaccine platforms (recombinant protein, autologous tumor cells, gene expression vectors) have been used to elicit antitumor CTL (1, 2). We have been investigating the utility of recombinant adenovirus (Ad)[3] vaccines and dendritic cell (DC)-based vaccines for tumor treatment (3, 4). Our prototypic Ad vaccine (Adhgp100) encodes the human melanoma Ag glycoprotein, gp100, which is a tissue-differentiation Ag expressed by both normal melanocytes and melanoma cells in humans and mice. Two modalities for delivery were evaluated: 1) direct injection of virus and 2) a cellular vaccine composed of DC infected with Adhgp100 in vitro (DC/Adhgp100). The human homologue of gp100 (hgp100) was used for these experiments because our previous experience determined that vaccination with murine gp100 (mgp100) did not induce any measurable immunity. The use of xenogenic Ags (homologous proteins from different species; hgp100 in this case) has proven highly effective in overcoming self-tolerance and inducing protective immunity against weakly immunogenic tumor Ags. Indeed, vaccination with DC/Adhgp100 generated protective immune responses against B16F10 in 80% of vaccinated hosts whereas immunization by direct injection of Adhgp100 provided little, if any, protective effect. These results suggested that DC might act as an adjuvant to enhance the immunogenicity of Adhgp100. Selective depletion of T cell populations in vivo indicated that tumor rejection was dependent on $CD4^+$ T cells whereas, contrary to our expectations, $CD8^+$ T cells were not involved in tumor rejection (4). Concomitantly, another group using a different melanoma Ag, TRP-2, also demonstrated that DC-based vaccines for melanoma elicited protective $CD4^+$ T cells (5). These observations raised the possibility that immunization with the DC vaccine may elicit an effector $CD4^+$ T cell response that cannot be engaged by virus alone. However, it remains unclear why the vaccines did not also induce $CD8^+$ T cell-dependent antitumor immunity.

The absence of $CD8^+$ T cell-dependent immunity may reflect a feature of the immune system that biases toward $CD4^+$ T cell responses when self-Ags are used. This possibility seems unlikely because immunization with hgp100 can produce $CD8^+$ T cell-dependent immunity in other models (6). Another possible explanation is that $CD8^+$ T cell response generated by xenogenic immunization with hgp100 was not sufficient to reject the mouse B16F10 melanoma, possibly due to inadequate processing of gp100 in B16F10 and low levels of MHC class I. To address these questions in the present study, we analyzed the $CD8^+$ T cell response following immunization with gp100 in relation to reactivity against defined MHC class I-restricted epitopes from hgp100 ($hgp100_{25–33}$; KVPRNQDWL) and from mgp100 ($mgp100_{25–33}$; EGSRNQDWL) (7). To correlate the presence of gp100-specific CTL with protective immunity, vaccinated animals were challenged with either 1) the mouse B16F10 melanoma cell line that

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[3] Abbreviations used in this paper: Ad, adenovirus; DC, dendritic cell; h, human; m, murine.

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naturally expresses mgp100, 2) a transfected subline that overexpresses hgp100 (B16F10-hgp100), or 3) a B16F10 line that has been reconstituted with the TAP-1 gene (B16F10-TAP1) to enhance processing of mgp100.

Materials and Methods

Animals, viruses, and cell culture

Six- to eight-week-old C57BL/6 mice were obtained from Charles River Breeding Laboratories (Wilmington, MA). Adhgp100 is an E1/E3-deleted Ad that expresses the full-length hgp100 gene. AdBHG is an E1/E3-deleted virus that expresses no transgene. Ad vectors were propagated in 293 cells and purified on a CsCl gradient as previously described (8). C57BL/6 mouse-derived thymoma EL-4 and melanoma B16F10 cell lines were cultured in RPMI 1640 medium supplemented with 10% FBS, 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. B16F10 stably transfected with the rat TAP-1 (B16F10-TAP1) (9), B16F10 cells stably transfected with hgp100 (B16F10-hgp100) (6), and their transfectant control (B16F10-neo) were cultured in the presence of G418 (800 μg/ml; Sigma-Aldrich, St. Louis, MO) to maintain transgene expression.

Peptides

The D^b-binding peptides of hgp100 ($hgp100_{25–33}$; KVPRNQDWL) and mgp100 ($mgp100_{25–33}$; EGSRNQDWL) have been previously described by Overwijk et al. (7). The hgp100 peptide exhibits 100-fold greater affinity for the D^b molecule than the murine peptide due to differences in the three amino acids (7). A D^b-binding peptide from the lymphocytic choriomeningitis virus glycoprotein (p33; KAVYNFATM) was used as a negative control. All of the peptides were purchased from Dalton Chemical Laboratories (Toronto, Ontario, Canada), dissolved in distilled water, and stored at −20°C.

Preparation of bone marrow DC and infection with Ad vectors

Bone marrow-derived DC were cultured as previously described (4). On day 5, cultured DC were infected with Ad vectors at a multiplicity of infection of 100 PFU per cell for 2 h. Cells were then washed three times with PBS and injected into mice.

Immunization and tumor challenge

Mice were immunized s.c. with 1×10^6 Ad-transduced DC or intradermally with 1×10^8 PFU Ad vector alone. Fourteen days later, immunized animals were challenged by s.c. injection with B16F10-hgp100 (1×10^5) or B16F10-TAP1 (2×10^4 or 1×10^5) cells. In vivo depletion of T cell subsets was performed using the following mAbs: GK1.5 (anti-CD4; American Type Culture Collection, Manassas, VA) or 53-6.72 (anti-CD8; American Type Culture Collection). Ascites fluid (100 μl) for each mAb was injected (i.p.) 2 days before tumor challenge and then continued every third day until unimmunized animals developed palpable tumors. Tumor size was monitored daily and measured twice a week.

Intracellular cytokine staining

For staining of intracellular IFN-γ, splenocytes were harvested from mice 14 days after immunization with the DC/Ad or Ad vaccines. Cells were incubated for 6 h in 24-well plate at a concentration of 2×10^6 cells/well in 2-ml complete medium with 1 μg/ml indicated peptides and 0.5 mg/ml brefeldin A (Sigma-Aldrich). The concentration of peptides was chosen based on our dose-dependence studies in which 1 μg/ml hgp100 or mgp100 peptide could stimulate the maximal IFN-γ release by $CD8^+$ T cells from Adhgp100-immunized mice (data not shown). Cells were stained for intracellular IFN-γ using the Cytofix/Cytoperm kit (BD PharMingen, San Diego, CA) as specified by the manufacturer. Briefly, stimulated cells were washed twice, Fc receptors were blocked by incubation with rat anti-mouse CD16/CD32 (Fc Block; BD PharMingen) for 20 min, and the cells were stained with FITC-labeled rat anti-mouse CD8 (clone 53-6.7; BD PharMingen) for 30 min. After fixation and permeabilization for 20 min, the cells were stained with PE-labeled rat anti-mouse IFN-γ Ab (clone XMG1.2; BD PharMingen) or an isotype control Ab (rat IgG1) for 30 min and then analyzed by flow cytometry.

Cytotoxicity assays

Spleens were harvested 14 days after immunization. For Ag-specific CTL assays, EL-4 cells pulsed with hgp100 or mgp100 peptide (1 μg/ml for 1 h at 37°C) were used as targets. Spleen cells were stimulated with target cells for 5 days at a 30:1 ratio, and effector cells were harvested and mixed with ^{51}Cr-labeled target cells (B16F10 and its transfectants; B16F10 or EL-4 pulsed with gp100 peptides) at various ratios. Percentage of specific ^{51}Cr release was evaluated as [(cpm experimental − cpm background)/(cpm maximum − cpm background) × 100%].

Results

Based on our previous observations that protection against the mouse melanoma B16F10 using gp100 as a target Ag could only be achieved with a DC-based vaccine and required a $CD4^+$ effector cell (4), we wanted to determine whether this mechanism was a reflection of the tumor cell line or the vaccination platform.

We first sought to determine whether the lack of $CD8^+$ T cell-dependent antitumor immunity resulted from the inability of the DC/Adhgp100 or Adhgp100 vaccine to elicit such a response. To characterize the $CD8^+$ T cell response following immunization with DC/Adhgp100 or Adhgp100, we measured CTL effector function using both Cr release assays to monitor lytic activity and intracellular cytokine staining to quantify the Ag-specific $CD8^+$ T cells. As shown in Fig. 1, both immunization strategies produced robust lytic activity against EL-4 cells pulsed with an immunodominant D^b-binding peptide derived from hgp100 ($hgp100_{25–33}$), but not EL-4 cells alone, suggesting that both DC/Ad and Ad could effectively engage $CD8^+$ CTL. Although DC/Adhgp100 consistently elicited a higher level of $hgp100_{25–33}$-specific CTL than Adhgp100 in repeated experiments, the difference could be related to the in vitro restimulation conditions. To obtain a direct measure of peptide-specific $CD8^+$ CTL in vaccinated mice, freshly isolated splenocytes were stimulated briefly (6 h) with $hgp100_{25–33}$ and the frequency of IFN-γ-secreting, $CD8^+$ T cells was measured by flow cytometry. An example of the results from the flow cytometry is provided in Fig. 2; both DC/Adhgp100 and Adhgp100 could elicit $hgp100_{25–33}$-specific $CD8^+$ T cells whereas only background IFN-γ production was observed in cultures containing a control D^b-binding peptide, p33. Interestingly, in repeated experiments, the response elicited by Adhg100 was 4-fold greater than the response produced by the DC/Adhgp100 vaccine (4.9 ± 1.4% vs 1.2 ± 0.4% of total $CD8^+$ T cells, respectively; Fig. 2 and data not shown).

Given that both vaccination platforms can effectively elicit $CD8^+$ T cell immunity, the absence of $CD8^+$ T cell antitumor responses in the B16F10 model may be due to the resistance of B16F10 cells to CTL killing. To verify that $CD8^+$ CTL elicited by both Ad and DC/Ad vaccines could protect against growth of B16F10 tumors, immunized mice were challenged with a stably transfected line of B16F10 that expresses hgp100 (B16-hgp100). As negative controls for the vaccination protocol, mice were also

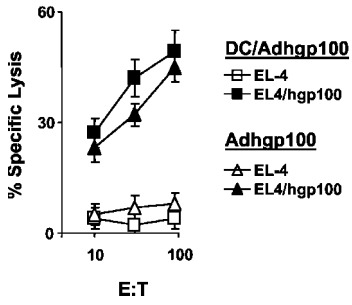

FIGURE 1. Immunization with DC/Adhgp100 or Adhgp100 stimulates CTL activation against hgp100 peptide. C57BL/6 mice were immunized with DC/Adhgp100 or Adhgp100. Fourteen days later, splenocytes were harvested from immunized animals and stimulated with hgp100 peptide-pulsed EL-4 cells for 5 days. The restimulated effectors were analyzed for cytolytic activity against hgp100 using a ^{51}Cr release assay. Data are representative of three experiments.

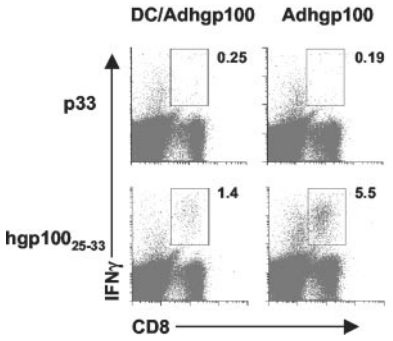

FIGURE 2. Immunization with DC/Adhgp100 or Adhgp100 elicits hgp100-specific IFN-γ secretion by $CD8^+$ T cells. C57BL/6 mice were immunized with DC/Adhgp100 or Adhgp100. Fourteen days later, freshly isolated splenocytes were restimulated with either hgp100 or p33 peptide and assayed for IFN-γ production on gated $CD8^+$ T cells. Numbers in FACS plots refer to $CD8^+$ IFN-γ^+ cells as a percentage of the total $CD8^+$ T population. Data are representative of four experiments.

immunized with AdBHG (an E1, E3-deleted Ad with no transgene) or DC/AdBHG. An additional group of sham-treated mice (PBS) were included as a positive control for tumor growth. As shown in Fig. 3*A*, the PBS group rapidly developed tumors in 10–15 days following B16F10-hgp100 challenge. Vaccination with AdBHG or DC/AdBHG did not provide any protective benefit. In contrast, mice vaccinated with DC/Adhgp100 or Adhgp100 alone were completely protected for the entire experimental period (60 days). $CD8^+$ and $CD4^+$ T cells were depleted before tumor inoculation to identify the effector population responsible for protective immunity. In vivo depletion of $CD8^+$ T cells starting 2 days before tumor challenge abrogated protective immunity whereas $CD4^+$ T cells did not seem to be required (Fig. 3*B*). These in vivo data demonstrate that both Ad and DC/Ad vaccines can elicit a $CD8^+$ T cell-dependent antitumor response, as had been initially anticipated.

One explanation for the failure of the $CD8^+$ T cells to clear the B16F10 tumor is that they may recognize only the human epitope but not the corresponding murine epitope. In Fig. 4*A*, freshly isolated splenocytes from Adhgp100- and DC/Adhgp100-vaccinated mice were restimulated with mgp100$_{25–33}$ and assayed for IFN-γ production by flow cytometry. Both immunization protocols elicited $CD8^+$ T cells reactive against the murine epitope. Although the number of $CD8^+$ T cells reactive against mgp100 was, on

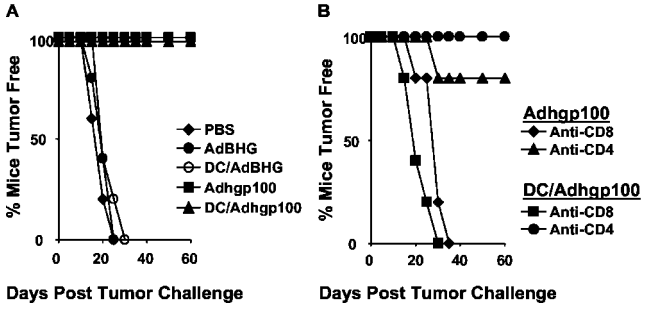

FIGURE 3. Both DC/Adhgp100 and Adhgp100 can elicit a CD8-dependent antitumor immunity against B16F10 expressing hgp100. *A*, C57BL/6 mice were immunized with DC/Adhgp100 or Adhgp100, challenged with 1×10^5 B16F10-hgp100 cells 14 days after immunization, and monitored for the onset of tumor formation. *B*, Ab depletion was initiated 2 days before tumor challenge and then every third day until most control animals (PBS-injected mice) developed palpable tumors. The results are representative of two experiments with five mice for each group.

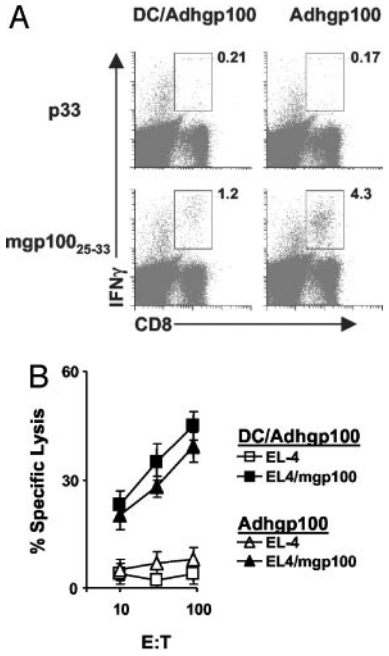

FIGURE 4. $CD8^+$ T cells activated by DC/Adhgp100 or Adhgp100 recognize mgp100 peptide. *A*, C57BL/6 mice were immunized with DC/Adhgp100 or Adhgp100. Fourteen days later, freshly isolated splenocytes were restimulated with either mgp100 or p33 peptide and assayed for IFN-γ production on gated $CD8^+$ T cells. Numbers in FACS plots refer to $CD8^+$ IFN-γ^+ cells as a percentage of the total $CD8^+$ T population. *B*, Splenocytes harvested from immunized animals were stimulated with mgp100 peptide-pulsed EL-4 cells for 5 days and the restimulated effectors were analyzed for cytolytic activity against mgp100 using a ^{51}Cr release assay. Data are representative of three experiments.

average, 25% lower than the number reactive against hgp100 (compared with Fig. 2), these modest differences cannot explain the lack of $CD8^+$ T cell-mediated tumor rejection. In agreement with the intracellular cytokine staining, CTL from hgp100-immunized mice exhibited a robust cytolytic activity against mgp100$_{25–33}$-pulsed EL-4 cells (Fig. 4*B*). Another possible explanation for the failure of $CD8^+$ T cells to clear B16F10 tumors is that the levels of MHC class I on the surface of the B16F10 are too low to adequately present mgp100 epitopes for CTL recognition. CTL from Adhgp100 and DC/Adhgp100 immunized mice failed to lyse B16F10 in chromium release assays, however, this was not due to low levels of MHC class I because up-regulation of MHC class I presentation by treatment of B16F10 for 24 h with IFN-γ (>90% of cells were MHC class I-positive, data not shown) did not increase their susceptibility to CTL killing in vitro (Fig. 5, *A* and *B*). As further evidence that MHC class I levels on B16F10 are sufficient to mediate CTL-dependent lysis, B16F10-hgp100 cells were demonstrated to be susceptible to lysis by gp100-specific CTL (Fig. 5, *A* and *B*). Thus, the failure of gp100-specific CTL to lyse B16F10 was not due to the absence of MHC class I-peptide presentation. A remaining possibility is that there is inadequate processing of mgp100 for MHC class I presentation. Indeed, B16F10 cells pulsed with either hgp100$_{25–33}$ or mgp100$_{25–33}$ were susceptible to lysis by gp100-specific CTL (Fig. 5, *C* and *D*). Based on these observations, the failure of Adhgp100 and DC/Adhgp100 vaccines to elicit $CD8^+$ T cell-mediated clearance of B16F10 appears to be a result of insufficient processing of MHC class I peptides from mgp100.

B16F10 have profound deficiencies in the Ag-processing machinery for MHC class I presentation, including defects in TAP-1

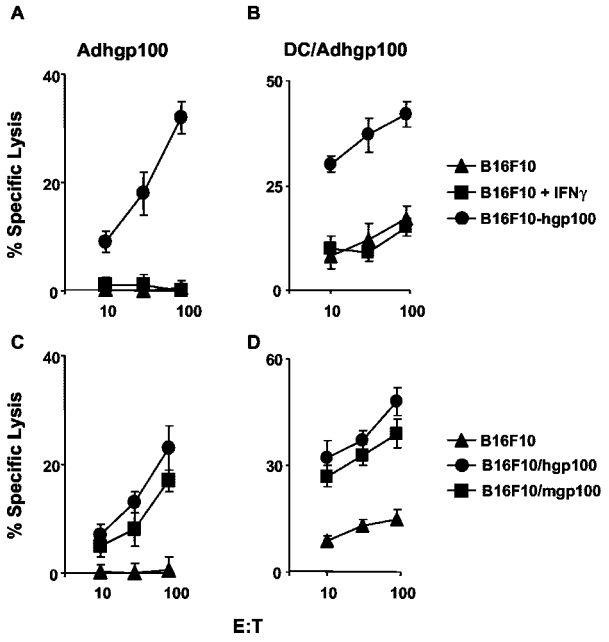

FIGURE 5. Ag-pulsed or -transfected B16F10 cells are susceptible to lysis by relevant CTL. C57BL/6 mice were immunized with DC/Adhgp100 or Adhgp100, and splenocytes were harvested for in vitro CTL assays 14 days later. *A–B*, Splenocytes from immunized mice were assayed for the presence of cytolytic activity against gp100 using following targets: B16F10 (▲), B16F10 treated with IFN-γ (■), and B16F10-hgp100 (●). *C–D*, Splenocytes from immunized mice were assayed for gp100-specific cytolytic activity using B16F10 (▲) and B16F10 pulsed with hgp100 peptide (●) or mgp100 peptide (■) as targets. The results are representative of two experiments.

expression (10). We have previously demonstrated that forced expression of TAP-1 can restore MHC class I expression and $CD8^+$ T cell presentation in cell lines defective in TAP-1/TAP-2 expression (9). Indeed, B16F10 cells stably transfected with the TAP-1 gene (B16F10-TAP1) display greatly increased MHC class I expression (data not shown). We have also demonstrated previously, that expression of TAP-1 could restore the peptide processing in cell lines defective in various components of the MHC class I Ag-processing machinery (9). Based on our previous experiences, we hypothesized that TAP-1 expression would restore presentation of mgp100-derived epitopes and render B16F10 susceptibility to hgp100-induced $CD8^+$ T cells. In support of this hypothesis, in vitro CTL analysis demonstrated that B16F10-TAP1 but not a control line (B16F10-neo) could be directly killed by gp100-specific

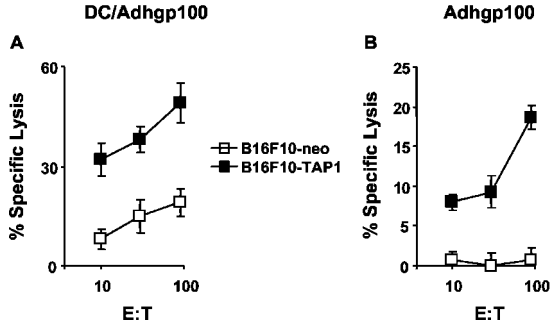

FIGURE 6. TAP-transfected B16F10 cells are susceptible to lysis by gp100-specific CTL. C57BL/6 mice were immunized with DC/Adhgp100 (*A*) or Adhgp100 (*B*) and 14 days later, splenocytes were assayed for the presence of cytolytic activity against B16F10-TAP1. The results are representative of two experiments.

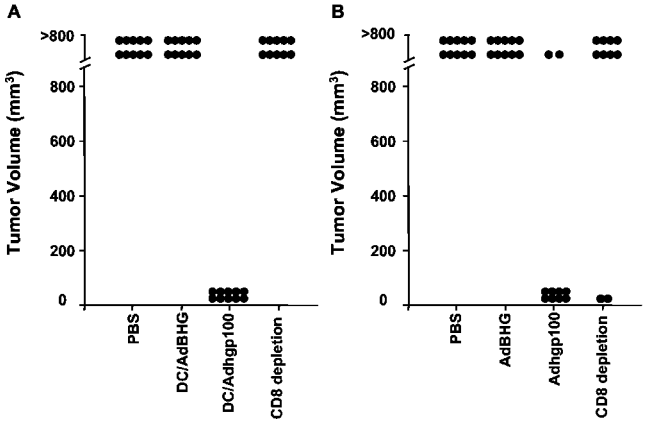

FIGURE 7. Both DC/Adhgp100 and Adhgp100 can elicit a CD8-dependent antitumor immunity against TAP-transfected B16F10 cells. C57BL/6 mice were immunized with DC/Adhgp100 (*A*) or Adhgp100 (*B*) and challenged with 1×10^5 B16F10-TAP1 cells 14 days after immunization. Tumor size was estimated by determining the longest diameter and average width and calculating the volume assuming a prolate spheroid. Ab depletion was initiated 2 days before tumor challenge and then every third day until most control animals (PBS-injected mice) developed palpable tumors.

CTL (Fig. 6). Most importantly, mice immunized with Adhgp100 and DC/Adhgp100 were resistant to challenge with B16F10-TAP1 cells and depletion of $CD8^+$ T cells before challenge abrogated protective immunity confirming a $CD8^+$ T cell-dependent mechanism for antitumor immunity when B16F10-TAP1 cells are used as challenge for gp100-immunized mice (Fig. 7). Thus, the absence of $CD8^+$ T cell-dependent antitumor immunity in our previous studies appears to be a consequence of defective Ag processing leading to inadequate expression of MHC class I-mgp100 complexes on the surface of the B16F10 cells.

The lack of $CD4^+$ T cell-mediated protection in DC/Adhgp100-immunized mice (Figs. 3 and 7) seemed inconsistent with our previous results in which the rejection of B16F10 cells was solely dependent on $CD4^+$ T cells (4). One possible explanation for this discrepancy is that transfection of the B16F10 cells alters their susceptibility to $CD4^+$ T cell-mediated clearance. Alternatively, the dose of B16F10-TAP1 that we used in these studies (10^5 cells) may be too large to be controlled by $CD4^+$ T cells. In our previous work, we used a challenge dose of 2×10^4 B16F10 cells and protection was lost when we increased the dose to 10^5 B16F10. To determine whether the B16F10-TAP1 were susceptible to $CD4^+$ T

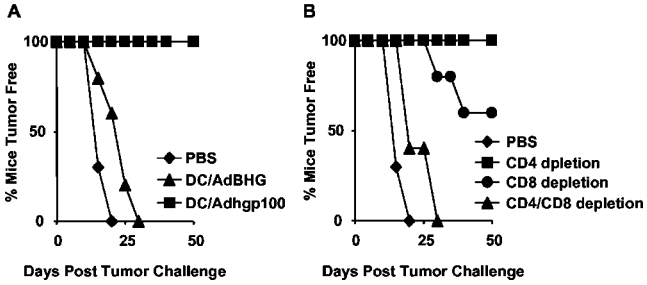

FIGURE 8. B16F10-TAP1 cells can be rejected by $CD4^+$ T cell-mediated immunity. *A*, C57BL/6 mice were immunized with DC/Adhgp100 or DC/AdBHG. Fourteen days later, mice were challenged with 2×10^4 B16F10-TAP1 cells and monitored for the onset of tumor formation. *B*, Ab depletion of T cells in immunized animals was initiated 2 days before tumor challenge and then every third day until most control animals (PBS-injected mice) developed palpable tumors. Data presented are summarized from eight to ten mice at each group.

cell-mediated clearance, mice were immunized with the DC/Adhgp100 vaccine and challenged with 2×10^4 B16F10-TAP1 cells. $CD4^+$ and $CD8^+$ T cells were depleted before tumor challenge. As shown in Fig. 8, protection in mice immunized with DC/Adhgp100 was abrogated only by simultaneous depletion of $CD4^+$ and $CD8^+$ T cells, indicating that either $CD4^+$ or $CD8^+$ T cells could independently mediate immune responses against B16F10-TAP1 cells. Furthermore, these results indicate that the $CD8^+$ T cell response induced by the DC/Ad vaccine can mediate protection against a larger tumor burden than the corresponding $CD4^+$ T cell response when the tumor cells express the relevant MHC class I-peptide complexes.

Discussion

In our previous experiments, we determined that the DC/Adhgp100 vaccine could elicit protective immunity against B16F10 but direct injection of Adhgp100 alone could not (11). Furthermore, the protective immunity produced by the DC vaccine was $CD4^+$ T cell-dependent (4). This observation prompted us to hypothesize that the DC vaccine may have a unique ability to activate $CD4^+$ effector T cells and that B16F10 may be resistant to gp100-specific CTL. However, other research has demonstrated that B16F10 can be cleared via $CD8^+$ T cell-dependent mechanisms and that DC vaccines can elicit $CD8^+$ CTL (12–14). Another possibility for our observation was that $CD8^+$ T cell responses against gp100, which is a normal nonmutated melanocyte protein, might be restricted by some form of tolerance and that the DC vaccine could overcome this tolerance through activation of $CD4^+$ T cells.

The studies we have described demonstrate that both the Ad and the DC/Ad vaccines can effectively generate $CD8^+$ T cell responses against gp100. Moreover, the $CD8^+$ T cell response generated by injection of Ad alone was greater than the response produced by the DC/Ad vaccine. Thus, the failure of the Adhgp100 vaccine to protect against B16F10 challenge was not due to an inability to engage $CD8^+$ T cell effectors. Using CTL from the spleens of hgp100-immune mice, we also demonstrated that B16F10 pulsed with a peptide epitope from mgp100 were sensitive to CTL lysis indicating that resistance to mgp100-specific CTL was not due to lack of available MHC class I. Finally, enhancement of the MHC class I processing machinery by forced expression of TAP1 in B16F10 sensitized these cells to $CD8^+$ T cell-dependent antitumor immunity following immunization with gp100. These data indicated that resistance of B16F10 to gp100-specific CTL is due to a lack of appropriate epitope presentation. Therefore, our previous observation of $CD4^+$ T cell-dependent antitumor immunity in this model is a reflection of the B16F10 tumors themselves and not the vaccine platform or the Ag.

These results have important implications for the development of viable cancer vaccination approaches. The failure of peptide-specific CTL to recognize tumors has been documented in both animal studies and clinical trials. Zaks and Rosenberg (15) demonstrated that immunization of patients with peptide 369–377 from the breast cancer Ag HER-2/neu yielded peptide-specific CTL that failed to recognize HER-2/neu-positive tumors. Likewise, another clinical study showed that T cell precursors reactive against an HLA-A0201-restricted telomerase-derived peptide (p540–548) were present in melanoma patients and these T cells exhibited lytic activity against HLA-A0201-positive cells pulsed with peptide or transiently transfected with a gene encoding the minimal epitope. However, the peptide-specific CTL were unable to recognize autologous tumor cells expressing human telomerase unless the antigenic peptide was added exogenously (16). Consistent with these observations, our data indicated that although immunization with either DC/Adhgp100 or Adhgp100 generated a potent CTL response against the 25–33 epitope from mgp100, these CTL were not able to lyse B16F10 cells that stain strongly for gp100 protein by immunohistochemistry (Y. Wan, unpublished observation).

Our results suggest that mgp100 peptide is not efficiently processed for MHC class I presentation in B16F10. Reduced generation of an antigenic peptide in tumor cells as a result of inefficient processing or defective transport via the TAP complex has been observed in many tumor types from both human and mice (17–20). Therefore, as demonstrated in the present work, developing tumor vaccines that only engage $CD8^+$ T cell effectors will be limited by the phenotype of the tumor cell itself. Our data demonstrate that the advantage of DC-based vaccination may not be due to an adjuvant quality of the DC but rather the ability of the DC to effectively activate a population of antitumor $CD4^+$ effectors. Whereas the nature of the $CD4^+$ T cell effector is still being elucidated, a number of groups have suggested that these cells mediate their antitumor activity through release of IFN-γ and recruitment of innate effectors such as eosinophils (21–24).

Previous work on DC-based cancer vaccines has suggested that DC can act as a "natural adjuvant" to enhance antitumor immunity mainly by promoting Ag-specific CTL responses (25). Particularly, when the tumor Ag is a weakly immunogenic protein, using DC as a vaccine carrier may be crucial to raise the amount of peptide-MHC class I complex to surpass the TCR threshold and to facilitate $CD4^+$ T cell help in maximizing $CD8^+$ CTL activation (26, 27). Our data add another important dimension to the utility of DC vaccines, and indicate that genetically modified DC can engage $CD8^+$ T cell-independent antitumor response that becomes critical when the CTL epitope is not adequately expressed by tumor cells. This has significant implications for cancer vaccine design, as a single vaccination platform may be able to target both CTL epitope-positive and epitope-negative tumors.

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