Genetically Modified Dendritic Cells Prime Autoreactive T Cells through a Pathway Independent of CD40L and Interleukin 12: Implications for Cancer Vaccines

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ABSTRACT

Genetic immunization through ex vivo transduction of dendritic cells has been suggested as an effective approach to enhance antitumor immunity by activating both CD4+ and CD8+ T cells. Immunizing mice with dendritic cells transduced with an adeno virus expressing the human melanoma antigen glycoprotein 100 (DCAdhgp100) as a cancer vaccine, we demonstrated complete protective immunity and a potent CTL response against melanomas expressing murine glycoprotein 100 in a CD4+ cell-dependent manner. Surprisingly, however, effective tumor rejection was not the result of cooperation between CD4+ and CD8+ T cells. Protective immunity was completely lost when CD4+ cells were depleted immediately before tumor challenge, whereas it was unaffected by removal of CD8+ cells, establishing a principal role for CD4+ cells in the effector phase of tumor rejection. Neither protective immunity nor CTL generation in this model required interleukin 12, in spite of high levels of IFN-γ secretion by tumor-reactive T cells. Most notably, the DCAdhgp100 vaccine could elicit protective antitumor CD4+ cells in the absence of CD40 ligand, although it does not bypass the need for CD40-mediated signals to generate melanoma-reactive CTLs. Thus, in contrast to the current thinking that the optimal cancer vaccine should include determinants for both CD4+ and CD8+ cells, the potency of the DCAdhgp100 vaccine appears to be a result of its ability to directly prime autoreactive CD4+ cells through a process that does not require interleukin 12 and CD40 signals.

INTRODUCTION

Cancer vaccines offer the promise of a new generation of therapies using the immune system to cure tumors. This approach is expected to have the capacity to eliminate metastatic disease without the side effects of current cytotoxic approaches. Evidence that the immune system is stimulated by determinants expressed on malignant tissue comes from studies with tumor-reactive T-cell lines generated from the tumor-infiltrating lymphocytic populations and peripheral blood of diseased patients. These T-cell lines have been used to identify and clone antigenic targets on tumors, providing a foundation for the development of cancer vaccines (1, 2). One aim of vaccinologists is to build on the preexisting immune repertoire and raise the T-cell reactivity to a level capable of tumor rejection. Unfortunately, many potential vaccine targets in tumors are nonmutated self-proteins, and, as such, the immunization process is limited by self-tolerance (3–5).

Two similar strategies have been developed to overcome self-tolerance to tumor antigen: (a) immunization with heteroclitic peptides (through protein engineering of key MHC/TCR contact residues; Ref. 6); and (b) xenoimmunization (using homologous proteins from different species; Refs. 7–9). Both strategies operate by activating T cells with low affinity for self-peptide using strong agonist peptide variants, which ultimately leads to cross-reactivity with the natural peptides expressed on the tumor cell. However, despite enhanced T-cell activation, tolerance to self-proteins, such as the melanoma antigen gp100,3 is not readily overcome. Genetic vaccination of mice with the xenoantigen, hgp100 generated cross-reactive CD8+ T cells responsive to either hgp100 or murine gp100. However, whereas immunized animals could resist challenge with tumors engineered to express hgp100, they eventually succumbed to tumor cells naturally expressing murine gp100 (8, 10–13). On the other hand, recent reports from our laboratory (14) and Kaplan et al. (15) demonstrated that vaccination using bone marrow-derived DCs genetically modified with an Ad expressing hgp100 (DCAdhgp100) could produce almost complete protective immunity in a CD4+ cell-dependent manner. These data suggested that the DC/Ad vaccine approach might activate an alternate set of autoreactive T cells, thereby offering a unique advantage for raising immunity against weak tumor antigens.

Until recently, most tumor vaccines have been designed to maximize the CTL response. New evidence, however, points to the central role of CD4+ T cells in directing both innate and adaptive antitumor immune responses (16–18). In fact, a recent report has demonstrated that immunization with a CD4+ T-cell epitope alone can effectively protect mice from virally induced tumors (19). Furthermore, in addition to providing “help” through paracrine cytokine secretion, CD4+ cells play a critical role in CTL priming by stimulating the antigen-presenting function of DCs through the interaction of CD40 and CD40L (20–22). Interestingly, virus infection of DCs could bypass the requirement of CD40L for CD8+ CTL activation, but activation of CD4+ T cells was still dependent on CD40L, indicating that CD40 signaling may be necessary for priming both CD4+ and CD8+ T cells (23, 24).

Using DCAdhgp100 immunization against B16 murine melanoma as a model, we have investigated the role of T cells, MHC presentation, IL-12 production, and CD40 signaling after immunization of genetically deficient mice. Our results indicate that the DC/Ad vaccine directly stimulates autoreactive CD4+ T cells, leading to tumor rejection through a pathway that is independent of CD8+ T cells and activation signals from IL-12 and CD40 ligation.

MATERIALS AND METHODS

Animals and Cell Culture. Six- to eight-week-old C57BL/6 and BALB/c mice were obtained from Charles River Laboratories (Wilmington, MA). CD8−/− and CD4−/− mice were kindly provided by Tak Mak (Ontario Cancer Institute, Toronto, Canada). IL-12 p40−/− mice were kindly provided by Jeanne Magram (Hoffmann-La Roche, Inc.). C57BL/6-B2m−/− (B2m-deficient, B2m−/−), B6, 129S-H2Kd/H2Kit−/− (MHC class II−/−), C57BL/6-Jsf/sf (CD40L−/−), and C57BL/6-Lyst−/− (beige) mice were purchased from Jackson Laboratories (Bar Harbor, ME). All the tumor cell lines were derived from C57BL/6 mice. B16F10 is a subclone of the spontaneous murine melanoma B16, MCA207 is a methylcholanthrene-induced fibrosarcoma, and EL4 is a lymphoma. B16F10 and MCA207 were cultured in DMEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin. EL4 cells were cultured in RPMI 1640 supplemented with the same additives as described above.

Received 1/7/00, accepted 4/17/00.

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3 The abbreviations used in this paper are: gp, glycoprotein; hgp100, human glycoprotein 100; DC, dendritic cell; Ad, adenovirus; β2m, β2 microglobulin; PKO, perforin knockout; IL, interleukin; CD40L, CD40 ligand; NK, natural killer; MAb, monoclonal antibody; ATCC, American Type Culture Collection; GM-CSF, granulocyte macrophage colony-stimulating factor; Th, T helper; NKT, natural killer T.
Adenoviral Vectors. Recombinant Adhgp100 and AdLacZ were provided by Genzyme (Framingham, MA). Both vectors were E1 deleted and E4 modified (removal of all open reading frames except orf6). AdLacZ contains the gene for *Escherichia coli* LacZ under control of the human cytomegalo-virus immediate early promoter (25). Viruses were propagated on 293 cells and purified by cesium chloride gradient centrifugation as described previously (26).

Preparation of Bone Marrow DCs and Infection with Ad Vectors. Bone marrow cells harvested from mouse femurs and tibias were cultured in 24-well plates (1 x 10^6 cells/well) in 1 ml of RPMI 1640 containing 10 ng/ml recombinant murine GM-CSF and 10 ng/ml recombinant murine IL-4 (kindly provided by Schering-Plough Research Institute, Kenilworth, NJ). Nonadherent cells were removed on day 2, and the remaining cells were fed with fresh RPMI 1640/GM-CSF/IL-4. On day 4, DCs were infected with Adhgp100 or AdLacZ at a multiplicity of infection of 100 per cell and placed in culture for another 24 h. Ad-transduced DCs were purified over metrizamide (>90% purity). No phenotypic or functional alterations (migratory and allostimulatory properties) were noted in DCs after Ad infection (data not shown).

Immunization. Mice were immunized with 1 x 10^6 Adhgp100-infected DCs in 200 μl of PBS injected s.c. in the hind flank. Control animals received either PBS or DCAdLacZ. Immunodepletion studies were completed using MAbs GK1.5 (anti-CD4; ATCC, Manassas, VA), 53-6.72 (anti-CD8; ATCC), and PK136 (anti-NK; ATCC). Hybridoma ascites fluid (100 μl) for each MAb was diluted in PBS (total volume, 500 μl) and injected i.p. MAbs were injected 2 days before vaccination and then injected every third day until day 14. Fourteen days after immunization, animals were challenged with 1 x 10^6 B16F10 cells by s.c. injection in the left hind flank. In some experiments, CD4 depletion was initiated 2 days before tumor challenge and then initiated every third day until most control animals (immunized with PBS and challenged with B16F10 cells) developed palpable tumors. To determine the antigen specificity of tumor rejection in vivo, immunized mice were challenged with MCA207 or EL4 tumor cells. Tumor size was monitored daily and measured twice a week in each group.

Cytotoxicity Assays. Splenocytes were harvested 14 days after immunization. B16F10 cells were used as target cells for gp100-specific CTL assays, and EL4 cells were used as a non-gp100-expressing control. Spleen cells were stimulated with target cells for 5 days at a 50:1 ratio, and effector cells were harvested and mixed with ^51^Cr-labeled target cells at various E:T ratios. Percentage of specific ^51^Cr release was evaluated as follows: (cpm experimental − cpm background)/cpm maximum × 100%.

Cytokine Assays. Splenocytes harvested from mice 14 days after immunization with DCAdhgp100 were cultured with irradiated B16F10 cells at a 50:1 ratio in RPMI 1640 supplemented with 10% fetal bovine serum. After a 72-h incubation, supernatants were analyzed for IFN-γ using ELISA kits from R&D Systems (Minneapolis, MN).

RESULTS

Antitumor Immunity after Immunization with DCs Transduced with Adhgp100 Is CD4⁺ Dependent. We have demonstrated previously that immunization of wild-type C57BL/6 mice with the DCAdhgp100 vaccine resulted in a potent anti-gp100-specific immune response capable of protection against B16F10 challenge (14). In this study, to evaluate the contribution of CD4⁺ cells in the protective response, T-cell subsets were depleted using specific antibodies either at the time of immunization (priming phase) or immediately before tumor challenge [effector phase (Fig. 1, A and B)]. Animals depleted of CD4⁺ cells during either phase experienced complete loss of tumor protection, demonstrating that CD4⁺ cells are critical for both priming and effector function of the gp100-specific immune response. On the other hand, CD8⁺ cells were dispensable in both cases (Fig. 1, A and B), indicating that classic CTLs were not required for tumor protection in this model. These observations were confirmed using genetically engineered mice lacking CD8⁺ cells (CD8⁻) or CD4⁺ cells [CD4⁻/CD8⁻ (Fig. 1C)]. As observed with the antibody depletion experiments, the absence of CD8⁻ cells had no effect on protection from B16 tumor, whereas loss of CD4⁺ cells completely abrogated the protective response. Thus, an autoreactive CD4⁺ cell is playing the central role in the observed response.

Specificity of the antitumor immune response (anti-gp100) generated by DCAdhgp100 vaccination was further defined by the out-

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*Fig. 1. Induction of antitumor immunity after DCAdhgp100 immunization is CD4⁺ dependent at both the priming and effector phases. Mice were injected s.c. with 1 x 10^6 DCs, challenged with a lethal dose of B16F10 cells 14 days after immunization, and monitored for the onset of tumor formation. A, antibody depletion was initiated 2 days before immunization (priming phase). B, antibody depletion was initiated the day before tumor challenge (effector phase). •, PBS-treated mice; ◆, mice treated with DCs transduced with AdLacZ; ■, mice treated with DCs transduced with Adhgp100; □, mice treated with DCs transduced with Adhgp100 and depletion of CD8⁻ cells; ○, mice treated with DCs transduced with Adhgp100 and depletion of CD8⁺ cells. Data are representative of three to seven independent experiments with four to five mice for each group. C, genetically deficient mice were injected s.c. with 1 x 10^6 DCs, challenged s.c. with 1 x 10^3 B16F10 cells 14 days after immunization, and monitored for the onset of tumor formation. ■, wild-type mice; ◆, CD8-deficient mice; ○, CD4-deficient mice. Data are representative of a minimum of three independent experiments.*

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growth of nonmelanoma tumors in immunized CD8\(^{−/−}\) mice. As shown in Fig. 2, only B16F10 melanoma tumors were rejected in DCAAdhgp100-vaccinated mice, whereas the growth of both MCA207 fibrosarcoma and EL4 lymphoma tumor cells was unaltered.

The natural processing of antigen by genetically modified DCs bypasses the need to identify MHC-specific epitopes from cancer antigens. An additional advantage of gene-based vaccines is that peptides from membrane-bound and secreted proteins are naturally targeted to the MHC class II complex. The gp100 protein has been localized to the cytoplasm, so it is unlikely that it traffics through the Golgi apparatus during processing. Therefore, it was of interest to determine how the protein epitopes were presented on MHC class II molecules for activation of CD4\(^{+}\) cells. Previous studies have demonstrated that immunization with genetically modified DCs results in antigen transfer to host DCs, which could lead to processing of antigen for the exogenous pathway of CD4\(^{+}\) activation (27).

To address this issue, we vaccinated mice with class II \(\beta\) DCs and observed complete loss of antitumor immunity (Fig. 3). Additionally, no protection was observed after immunization with allogeneic DCs (H-2\(^{b}\)). Thus, it appears that priming of autoreactive CD4\(^{+}\) cells by the DC/Ad vaccine is the result of direct presentation of tumor antigen by the adaptively transferred DCs and that antigen transfer in this model is an inefficient process for eliciting protective immunity against B16.

**Role of NK1.1\(^{+}\) Cells in Antitumor Immunity.** We observed a minor loss of protective immunity after \textit{in vivo} depletion of NK1.1\(^{+}\) cells (data not shown). This suggested that either DCs were involved in the protective response (in conjunction with CD4\(^{+}\) T cells) or that protective immunity may be related to the NKT subset that comprises both CD4\(^{+}\) NK1.1\(^{+}\) T cells and CD4\(^{−}\)NK1.1\(^{+}\) T cells. NKT cells represent an unusual subset of lymphocytes that can be activated by DCs (28) and have been shown to be involved in antitumor immune responses elicited by IL-12 (29). To exclude the possibility that NKT cells were responsible for the responses observed in our tumor model system, the efficacy of the DC vaccine was examined in both \(\beta\)m\(^{−/−}\) and class II \(\beta\)m\(^{−/−}\) mice. \(\beta\)m\(^{−/−}\) mice are NKT deficient due to an impairment in CD1 expression (30), whereas class II \(\beta\)m\(^{−/−}\) mice retain a diverse population of CD1-selected NKT cells (31). Both \(\beta\)m\(^{−/−}\) and class II \(\beta\)m\(^{−/−}\) mice were immunized with DCAAdhgp100 and challenged with B16F10 cells as described above. An additional group of \(\beta\)m\(^{−/−}\) mice was depleted of CD4\(^{+}\) cells during the course of immunization and challenge. In these studies, \(\beta\)m\(^{−/−}\) mice were completely protected against B16F10 challenge (Fig. 4A), whereas class II \(\beta\)m\(^{−/−}\) mice did not respond to DC immunization (Fig. 4B). Depletion of CD4\(^{+}\) T cells resulted in a complete loss of DC-induced protective immunity in \(\beta\)m\(^{−/−}\) mice, demonstrating that CD4\(^{+}\) cells were essential for tumor rejection and that these were not NKT cells (Fig. 4A).

To further characterize the possible involvement of NK1.1\(^{+}\) cells in this protection model, \textit{beige} mice that lack NK function were used as vaccine recipients (Fig. 4C). Whereas the \textit{beige} mice displayed a delay in tumor onset compared with wild-type animals, only 38\% of the mice were completely protected from tumor challenge in contrast to 100\% of wild-type mice. These data demonstrate that CD4\(^{+}\) cells play a central role in DCAAdhgp100-induced protection against B16, whereas NK cells play a secondary role, one that is likely directed by the CD4\(^{+}\) cells.

**Protective Immunity and Effector Cell Activation by Genetically Modified DCs Are IL-12 Independent.** IL-12 has been shown to play a critical role in DC function both as a paracrine factor to enhance CTL and Th1 cell maturation and as an autocrine factor for DC activation (32, 33). To further characterize the pathway of immune cell activation in this model, we used mice that were deficient in IL-12 production [IL-12 \(^{−/−}\) (34)]. IL-12 production from wild-type DCs was 0.5–1 ng/10\(^6\) cells/24 h, whereas production from IL-12 \(^{−/−}\) DCs was undetectable (data not shown). Both wild-type and IL-12 \(^{−/−}\) mice were largely protected from tumor challenge after vaccination with IL-12 \(^{−/−}\) DCAAdhgp100 (Fig. 5A). Intriguingly, vaccinated IL-12 \(^{−/−}\) mice displayed levels of CTL activity comparable with those of wild-type mice (Fig. 5B), and splenocytes from both wild-type and IL-12-deficient mice secreted equivalent amounts of IFN-\(\gamma\) after \textit{in vitro} stimulation (Fig. 5C; 13.6 ± 0.24 and 12.3 ± 0.18 ng/ml in 72 h, respectively). Thus, our vaccination approach can lead to protective immunity and can prime IFN-\(\gamma\)-secreting effector cells in an IL-12-independent fashion.

**Disruption of C40 Signaling Leads to Loss of CTL Function but Does Not Impair Protective Antitumor Immunity.** The observation that the DC/Ad vaccine is IL-12 independent may be a result of the highly mature state of our \textit{ex vivo} cultured DCs. High expression of costimulatory molecules or other T-cell activators by our DC preparations may bypass signaling pathways normally used by immature DCs that have recently migrated to the lymphatics carrying...
antigen from peripheral tissues. The CD40L–CD40 interaction is one pathway that appears to be critical to the activation of both CD4+ and CD8+ cells (20–24). Disruption of this pathway leads to suppression of allogeneic and protective immune responses in transplantation models and virus infection models in mice and higher primates (35–37). To evaluate the CD40 dependence of CD4+ T-cell and CTL activation, we immunized mice in which the CD40L gene was genetically inactivated (CD40L−/−). T cells in this model are unable to signal through CD40 and therefore should not be able to transmit a “danger” or “help” signal to the DCs. Consistent with previous reports demonstrating that CTL priming by DCs required CD40 ligation (33, 38), CD40L−/− mice were unable to mount a CTL response against B16 cells, demonstrating that viral infection of DCs using replication-deficient adenovirus was unable to overcome the dependence on CD40 for CTL activation (Fig. 6B). Most strikingly, however, the CD40L−/− mice were still fully protected against tumor cell challenge, demonstrating that stimulation of the autoreactive CD4+ T-cell response was independent of CD40 ligation (Fig. 6A). Further evidence that protective immunity was independent of cytolytic activity came from studies with perforin-deficient (PKO) mice (data not shown). In vitro studies demonstrated that the CTL activity in this model was perforin dependent.2 No CTLs were detectable in PKO mice, yet these mice were fully protected from tumor challenge. Therefore, the ability of our DC vaccine to overcome self-tolerance barriers appears to result from its ability to directly activate noncytolytic CD4+ T cells through MHC class II presentation using a stimulatory pathway that is CD40L and IL-12 independent.

DISCUSSION

The results presented here identify a principal role for CD4+ T cells in antitumor immunity not only as T helpers in the priming phase but as the major effector cell in the rejection phase. These data contrast with results of previous studies investigating genetic vaccination in which the protective immune response was incomplete and dominated by CD8+ cells (10–13).4 Whereas CD8+ CTLs were observed in our model, they do not seem to play an important role in the rejection of tumor burden. CTL independence was further confirmed by studies of DCAdhgp100-immunized CD40L−/− and PKO mice in which full protection was achieved in the absence of measurable cytolytic activity. Therefore, although ex vivo DC vaccination primes both CD4+ and CD8+ T cells, effective tumor rejection does not appear to be the result of a cooperative effect of CD4+ and CD8+ T cells. Rather, the central player appears to be the autoreactive CD4+ T cell. This finding differs from the current thinking that optimal tumor vaccines should contain both CTL and Th epitopes, allowing CD4+ Th cells to maximize CD8+ cell-mediated cytotoxicity (17). A new paradigm is emerging that dictates that a successful tumor vaccine directed at weak self-antigens must activate an effector CD4+ T-cell response for a strong protective effect (16–18). We show here that genetically engineered DCs offer a vaccine vehicle that matches these requirements.

A number of studies have demonstrated that CD4+ T cells exhibit effector functions independent of CD8+ CTLs. Immunization with whole tumor cells expressing GM-CSF CD4+ T cells was shown to mediate tumor rejection through activation of macrophages and eosinophils (39). Likewise, Greenberg et al. (40) and Mumberg et al. (18) showed that adoptive transfer of CD4+ T cells resulted in tumor protection related to macrophage stimulation and secretion of cytokines such as IFN-γ. Vaccination with a pox vector expressing tsp1/gp75 could break self-tolerance in a CD4+ cell-dependent manner, leading to autoimmune vitiligo (41). Our results are not only in accord with those prior reports regarding the important role of CD4 in antitumor immunity, but they also support the concept that activation of tumor-specific CD4+ T cells is a necessary step toward breaking the self-tolerance barrier. The fact that standard genetic immunization against gp100 only stimulated a CD8+ cell response and yielded

Fig. 4. NK but not NKT cells contribute to protective immunity. Mice were immunized with 1 × 106 DCs transduced with Adhgp100 and challenged with a lethal dose of B16F10 cells. A, β2m−/− mice were used as vaccine recipients. C, beige mice (NK deficient) were used as vaccine recipients. ●, PBC-treated mice; ○, mice treated with DCs transduced with Adhgp100; ▲, mice treated with DCs transduced with Adhgp100 and depleted of CD4+ T cells.
incomplete protection against challenge with B16 tumor seems to support this argument.

Whole cell tumor vaccines expressing the cytokine GM-CSF have demonstrated similar efficacy against the B16 melanoma as our DC/Ad vaccine (39). An important difference between the two immunization approaches is that the whole cell vaccine is dependent on cross-presentation of tumor antigen through host antigen-presenting cells, whereas antigen is presented by the DC/Ad vaccine directly to responding T cells, with little evidence of antigen transfer. This may also explain why standard approaches to genetic immunization were less efficient than the \textit{ex vivo} DC approach because the former may rely more strongly on cross-presentation than on direct transfection of DCs (42, 43). Antigen-presenting cells in cancer patients are often functionally impaired, which is likely to reduce the efficacy of protocols dependent on antigen transfer. \textit{Ex vivo} culture of DCs can restore their immunostimulatory functions, circumventing tumor-induced impairment of antigen presentation (44).

The \textit{in vivo} protective response to DCAdhgp100 vaccination in mice is dependent on MHC class II presentation but is independent of CD8\(^{+}\) cells, IL-12, and CD40. This is in contrast to the classic pathway of immune protection that is CD8\(^{+}\) and CD40 dependent and is often IL-12 dependent (45). Whereas this result can be interpreted in a number of ways, a simple explanation is that the DCAdhgp100 vaccine interacts directly with the autoreactive T-cell clone in a two-cell cluster, and, unlike CD40-mediated activation, the responding T cell does not require an additional signal from a third-party cell. Interestingly, CTL activation in our model is CD40L and CD4\(^{+}\) dependent, indicating that the three-cell pathway (Th-DC-CTL) is functional in our model, but this pathway is not used to achieve protective immunity to gp100. These observations appear paradoxical.

**Fig. 5.** CTL activation and protective immunity after DCAdhgp100 does not require IL-12. A, wild-type (■) and IL-12\(^{-/-}\) (□) mice were immunized with \(10^6\) DCAdhgp100 and \(10^6\) IL-12\(^{-/-}\) DCAdhgp100 cells, respectively. Immunized mice were then challenged with a lethal dose of B16F10 cells 14 days later. ●, PBS-treated mice. B, splenocytes from DC-immunized mice were restimulated with irradiated B16F10 for 5 days \textit{in vitro} and tested for lytic activity on B16F10 (squares) and EL4 (circles). Closed symbols, wild-type mice immunized with DCAdhgp100; open symbols, IL-12\(^{-/-}\) mice immunized with IL-12\(^{-/-}\) DCAdhgp100. C, supernatants from restimulated cultures in B were tested for the presence of IFN\(\gamma\) by ELISA. These data are representative of a minimum of three independent experiments with four to five mice per group. Similar results were obtained when wild-type mice were immunized with IL-12\(^{-/-}\) DCAdhgp100.

**Fig. 6.** Protective immunity but not CTL activation after DCAdhgp100 is independent of CD40L. A, wild-type (■) and CD40L\(^{-/-}\) (□) mice were immunized with \(10^6\) DCs transduced with Adhgp100 and challenged with a lethal dose of B16F10. ●, PBS-treated mice. B, splenocytes from DC-immunized mice were restimulated with irradiated B16F10 for 5 days \textit{in vitro} and tested for lytic activity on B16F10 (squares) and EL4 (circles). Closed symbols, wild-type mice immunized with DCs, open symbols, CD40L\(^{-/-}\) mice immunized with DCs. These data are representative of a minimum of three independent experiments with four to five mice per group.
in that the DCs in our inoculum are capable of directly activating autoreactive T cells but are not mature enough to activate CTLs in the absence of third-party cell signaling through CD40. Additional studies will be required to determine whether this paradox is true for non-self proteins as well.

These results have strong implications for the development of cancer vaccines and for understanding autoimmunity. Although previous studies of tumor immunity have always implicated a major role for CD8+ cells in the effector phase and an important role for CD4+ cells as helpers, studies in autoimmunity have demonstrated a predominant role of CD4+ cells as effectors (46–49). As the line between tumor immunity and autoimmunity becomes thinner, it is clear that we should consider overlaying paradigms with respect to developing new approaches for tumor immunotherapy (5). Thus, future design of cancer vaccines should focus on activating T-cell subsets already associated with autoimmune syndromes (i.e., CD4+ cells and Vp8.2+ cells; Refs. 50 and 51). Research in autoimmunity has suggested the possibility that there is a subset of CD4+ T lymphocytes that is primed by antigen-bearing DCs in an unusual manner, leading to “unchecked” activation [i.e., no requirement for a third-party cell (46)]. Whereas this approach may be beneficial for cancer vaccines, excessive use of this pathway could lead to unwanted autoimmune syndromes. Interestingly, we did not observe any evidence of autoimmune vitiligo in our studies in contrast to previous autoimmune syndromes. Interestingly, we did not observe any evidence of autoimmune vitiligo in our studies in contrast to previous autoimmune syndromes.

ACKNOWLEDGMENTS

We thank Duncan Chong, Xueya Feng, and Chunyan Li for expert technical assistance and Dr. Bruce Roberts for providing recombinant Ads.

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