Interleukin 18 Transfection Enhances Antitumor Immunity Induced by Dendritic Cell-Tumor Cell Conjugates

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ABSTRACT

Dendritic cell (DC)-based tumor vaccine represents a promising approach to the immunotherapy of malignant tumors. We prepared a novel type of DC-based vaccine, stable conjugates of DCs and EL4 cells transfected with cDNA of OVA (E.G7). Immunization with DC-E.G7 conjugates led to generation of T helper (Th) 1 cytokine-producing cells, antigen-specific CD4+ T cells, and strong antitumor immunity that is dependent on both CD4+ T cells and CD8+ T cells. To further increase the potency of the vaccine, interleukin 18-transfected DCs were used to prepare the IL18DC-E.G7 conjugates. Immunization with such conjugates significantly increased the production of Th1 cytokine-producing cells and the number of antigen-specific CD8+ T cells, as well as stronger antitumor immunity. Furthermore, the increased Th1 cytokine production and stronger antitumor effect were not observed in mice depleted of IFN-γ. These data indicated that DC-tumor cell conjugates are a potent tumor vaccine. Interleukin 18 can be administrated using gene-transfected cells and enhances antitumor immunity, which is mainly mediated by IFN-γ.

INTRODUCTION

Given the tremendous success of vaccination against infectious diseases, using tumor cells as immunogens to induce antitumor immunity is an attractive strategy to combat various human cancers. However, tumor cells themselves are usually poor immunogens. Possible reasons for this include down-regulation of MHC molecules, lack of costimulatory molecules, and inability to process and present antigen on cell surface (1). Numerous efforts have been made to transfect tumor cells with cDNAs of MHC molecules, costimulatory molecules, or particular cytokines to increase their immunogenicity and promote immune response (2). It has also been shown that a hybrid between tumor cells and antigen-presenting cells can induce strong antitumor immunity (3). Although many of these strategies yielded promising results in animal models, clinical trials in humans have been generally disappointing.

DCs are unique highly potent antigen-presenting cells capable of sensitizing naïve CD4+ T cells and CD8+ T cells (4). With the availability of isolation and bulk propagation of DCs in vitro, great efforts have been made to use DCs in various immunization strategies (5). Immunization with DCs pulsed with tumor antigens or antigen-driven peptides can generate tumor-specific immune responses and antitumor effects (6–9). Because only a limited number of tumor-associated or tumor-specific antigens have been identified and shown to be recognized by CTLs (10), tumor-derived protein extracts or RNA has been used as the source of antigen (11, 12). However, the antigen-presenting pathways in these situations and the efficiency in priming both CD4+ and CD8+ T cells are not well understood.

IL-18 was initially identified as a cytokine that facilitates the production of IFN-γ induced by endotoxin (13). It is an essential factor for IFN-γ production in response to microbial agents (14), and can act together with IL-12 in promoting the generation of IFN-γ-producing Th1 cells (15, 16). In IL-18-deficient mice, there are defects in the generation of Th1 response (17). These results indicate that IL-18 plays an essential role in inducing the Th1 response in vivo. Consistent with the notion that Th1 cells are involved in antitumor immunity, administrations of IL-18 with or without IL-12 have significant antitumor effects (18, 19). However, administration of IL-18 is associated with septic shock-like severe toxicity that prevents its application (20). Interestingly, it was found that immunization with tumor cells expressing IL-18 with or without IL-12 could generate a stronger antitumor effect than immunization with untransfected tumor cells (21), indicating that a vaccine expressing IL-12 and IL-18 may be used in inducing antitumor immunity.

To further understand the antigen-presenting process and the roles of CD4+ T cells and CD8+ cells in DC-based immunotherapy, we have constructed a model vaccine that consists of DCs and EL4 cells transfected with cDNA of OVA. We are able to detect antigen-specific T cells using peptide/MHC class I tetramer in this system. We showed here that the conjugates of DC-E.G7 could induce strong antitumor immunity. Furthermore, transfection of DCs with IL-18 cDNA enhanced induction of the Th1 response, antigen-specific CD8+ T cells, and antitumor immunity.

MATERIALS AND METHODS

Animals and Cell Lines. Male or female wild-type C57BL/6 mice or IL-4−/− (C57BL/6, H-2d) mice, 6–8 weeks of age, were obtained from SIPPR-BK Experimental Animal Co. (Shanghai, China) and housed in a pathogen-free facility for all experiments. E.G7 cells [EL4 cells transfected with cDNA of chicken egg albumin (OVA)] were kindly provided by Dr. E. Gilboa (Duke University, Durham, NC). Transformed human embryonic kidney 293 cells were purchased from American Type Culture Collection (Manassas, VA). All these cells and primary cells from mice were cultured in RPMI 1640 supplemented with penicillin (100 units/ml), streptomycin (100 μg/ml), 2-mercaptoethanol (5 × 10−3 M), and 10% FCS (complete medium).

Preparation of OVA Peptide/H-2Kb Tetramers. The 14- amino acid residues recognized by biotinylated enzyme Bir A (22, 23) were added to the COOH terminus of the extracellular region (residues 1–280) of H-2Kb heavy chain by PCR (5’-CTAGCTAGCAGGCTCACTCCTGAGG-3’; 5’-CGCGATCTTTAAGCTGATTCACACACCTCTTTGTCGATCCAGAT- ATGATGCAAGGATCAGTGGATGAGGAGGGCTC-3’). The amplified PCR product was cloned into pET 21d using NiI/BarHi sites (Novagen Inc., Madison, WI). Recombinant H-2Kb heavy chain with Bir A site and human β2-microglobulin were refolded together with the peptides corresponding to OVA residue 257–264 (SHINEKL) or TRP2 residue 180–188 (SVYDFFVWL) as described previously (8, 24). The H-2Kb–peptide–β2-microglobulin complexes were then purified through a Sephacryl S-200 column (Amer- sham Pharmacia Biotech, Piscataway, NJ) and biotinylated with Bir A enzyme (AVIDITY, Boulder, CO). The biotinylated complexes were further purified by gel filtration, and tetramerization was accomplished by mixing biotinylated
PK136 were used to deplete CD4+ T cells. McAb R4-6A2 was used to block endogenous IFN-γ produced by the DC-tumor cell conjugates, we transduced DCs with the gene encoding β-galactosidase (AdlacZ) and murine IL-18 (AdIL18) as described previously (11). These cells express CD80, CD86, CD40, and Iab on their surface. To pulse these cells with OVA257-264 peptides, they were incubated with 10 μg/ml peptides in the presence of human β2-microglobulin (10 μg/ml) at 37°C for 3 h. After washing twice with PBS, they were resuspended in PBS at 10^6 cells/ml and used for injection. DC-E.G7 and IL18DC-E.G7 conjugates were made by incubating DCs or IL-18 gene-modified DCs with E.G7 cells at a 6:1 ratio for 24 h in complete RPMI 1640 (27).

Immunization. The DC-E.G7 vaccine, the same number of DCs or E.G7 cells alone, DCs pulsed with peptides, or PBS was irradiated (20,000 rads) and injected s.c. into the right flank region of C57BL/6 mice, and the same immunizations were repeated once, a week later. Tumor challenge was initiated by injecting 10^6 E.G7 cells s.c. into the rear leg of the immunized mice one week after the last immunization. Tumor occurrence was observed twice a week, and a tumor injection site with a diameter of less than 0.5 cm was regarded as tumor free.

Immunotherapy with IL-18 Gene-modified DC-E.G7 Vaccine. For immunotherapy of established E.G7 tumors, tumor-bearing mice were established by s.c. inoculation of 10^5 E.G7 cells. IL-18 gene-modified DCs were incubated with E.G7 cells at a ratio of 6:1 for 24 h to form stable IL18DC-E.G7 vaccine (27). The IL18DC-E.G7 cell conjugate and various controls were injected s.c. into the flank region of the tumor-bearing mice 3 days after tumor immunization. The same therapy was repeated 1 week later. The tumor-bearing mice were sacrificed 7 days after the second immunotherapy, and tumor weights were determined.

In Vivo Depletion of Specific Cell Subsets. McAbs GK1.5, 2.43, and PK136 were used to deplete CD4+ T cells, CD8+ T cells, and NK cells, and McAb R4-6A2 was used to block endogenous IFN-γ in vivo. Antibodies (100 μg) were injected i.p. in 0.1 ml of PBS into each mouse at 4 days and 1 day before immunization or inoculation of tumors, and another three injections of antibodies were performed at 3-day intervals. Flow cytometry analysis of splenocytes and peripheral blood cells revealed that more than 95% of the targeted cells were depleted (data not shown).

Cytokine Production Assay. Spleen lymphocytes (2 × 10^6 cells/ml) from immunized or control mice were stimulated with irradiated E.G7 cells at a 10:1 ratio. Supernatants were collected after 24 h (for IL-2 assay), 48 h (for IFN-γ assay), or 72 h (for IL-10 assay). The amounts of cytokines were determined using ELISA kits from Endogen (Woburn, MA).

RESULTS

DC-E.G7 Conjugates Elicited Potent Antitumor Immunity that Depends on Both CD4+ and CD8+ T Cells. DCs and tumor cells are able to form stable conjugates that can be used as an immunogen to induce antitumor immunity (27). We have made such conjugates by culturing DCs with EL4 cells transfected with cDNA of OVA (E.G7). The majority (>95%) of the E.G7 cells in the culture were associated with DCs after 24 h, and the conjugates are stable after being washed twice with PBS containing 2% FCS (data not shown). C57BL/6 mice were immunized twice with DCs, E.G7 cells, DCs pulsed with OVA257-264 peptides, DCs pulsed with peptide TRP2180-188 or DC-E.G7 conjugates. Seven days after the second immunization, these mice were challenged with E.G7 tumor cells. As shown in Fig. 1A, all mice immunized with the DC-E.G7 vaccine remained tumor-free 3 months after the tumor challenge. The majority (80%) of the mice immunized with OVA257-264-pulsed DCs were also tumor free, whereas mice treated with PBS, DCs, or E.G7 alone and DCs pulsed with TRP2180-188 showed no significant protection against the inoculated E.G7 cells. Thus, DC-E.G7 conjugates are a potent vaccine that can induce efficient antitumor immunity.

To assess whether CD4+ and/or CD8+ T cells are involved in this antitumor immunity, we injected anti-CD4 or anti-CD8 antibodies to deplete CD4+ T cells or CD8+ T cells in mice. When CD4+ T cells were depleted before immunization of the mice with DC-E.G7 vaccine, only 10% of the mice remained tumor free 90 days after tumor challenge (Fig. 1B). In contrast, 70% of the mice were still tumor free when CD4+ T cells were depleted after immunization (Fig. 1B). Depletion of CD8+ T cells either before immunization or after tumor challenge significantly eliminated the protective effects of the DC-E.G7 vaccine (90 days after injection of tumor cells, 100% and 80% of mice died of tumors, respectively; Fig. 1B). Thus, both CD4+ and CD8+ T cells are required for effective antitumor immunity induced by DC-E.G7 conjugates. These data are consistent with the notion that CD4+ T cells play a critical role in the priming phase of antitumor immunity, whereas CD8+ T cells are critical effectors (28).

IL-18-transduced DC-E.G7 Vaccine Induces More Potent Antitumor Immunity. To further enhance the antitumor response induced by the DC-tumor cell conjugates, we transduced DCs with the cDNA of IL-18, which had been shown to promote IFN-γ production and Th1 differentiation (15–17). We tested the effects of this IL18DC-E.G7 vaccine in mice that had been injected with 10^5 E.G7 cells. Seven days after the second immunization, these mice were sacrificed to determine the weight of tumors. Although OVA257-264-pulsed DCs, DC-E.G7 conjugates, and LacZ gene-transfected DC-E.G7 vaccines effectively inhibited tumor growth (tumor weight, 0.36 ± 0.1,
0.28 ± 0.12, and 0.5 ± 0.16 gram, respectively) compared with mice treated with DCs alone (tumor weight, 1.55 ± 0.22 gram), IL18DC-E.G7 vaccine was significantly more potent (tumor weight, 0.08 ± 0.07 gram; Fig. 2A). The IL18DC-E.G7 vaccine was also found to be more potent than OVA257–264-pulsed DCs and the LacZ.DC-E.G7 vaccine when tumor-bearing mice were treated with each of these vaccines and then rechallenged with E.G7 tumor cells, indicating that specific antitumor immunity was induced after immunotherapy with IL-18 gene-modified DC-tumor conjugate vaccine (Table 1). The roles of CD4+ T cells and CD8+ T cells were further assessed by depleting them with anti-CD4 and anti-CD8 antibodies. As shown in Fig. 2B, depletion of CD4+ T cells or CD8+ T cells alone before immunotherapy significantly reduced the inhibition of tumor growth by the IL18DC-E.G7 vaccine. Pretreatment with anti-CD4 and anti-CD8 antibody completely abolished the effect of the vaccine on tumor growth. These results indicated that both CD4+ T cells and CD8+ T cells are required for antitumor immunity induced by the IL18DC-E.G7 vaccine.

**Induction of Antigen-specific CD8+ T Cells by DC-E.G7 Vaccines.** We made a PE-labeled H-2Kb/OVA257–264 tetramer that can specifically recognize OVA-specific CD8+ T cell clone (data not shown). Whereas few tetramer+ CD8+ T cells were detected in lymphocytes derived from mice treated with PBS or DCs pulsed with control peptide TRP2180–188, antigen-specific CD8+ T cells were found in mice immunized with either OVA257–264-pulsed DCs or DC-E.G7 vaccine (Fig. 3A). The average numbers from three independent experiments are shown in Fig. 3B. It is noteworthy that the percentage of antigen-specific CD8+ cells induced by DC-E.G7 is significantly higher than that induced by DCs pulsed with OVA257–264. In mice that already had E.G7 tumors, tetramer+ CD8+ T cells were detected after they were treated with IL18DC-E.G7, OVA257–264-pulsed DCs, or DC-E.G7 (Fig. 3C). The results from three independent experiments are summarized in Fig. 3D. In this case, a significantly higher number of tetramer+ CD8+ T cells were detected in mice immunized with IL18DC-E.G7 vaccine compared with mice immunized with DC-E.G7 or OVA257–264-pulsed DCs, indicating that the IL-18-transfected conjugates are more potent in inducing antigen-specific CD8+ T cells. We also examined the level of CTLs using 51Cr-labeled E.G7 cells as a target. CTL activities were significantly higher in lymphocytes from IL18DC-E.G7-immunized mice than in those from OVA257–264-pulsed DC- or DC-E.G7-immunized mice (data not shown). These results demonstrated that more tumor-specific CTLs were induced in tumor-bearing mice after stimulation with IL-18 gene-modified tumor vaccine.

**Cytokine Production after Immunotherapy with IL18DC-E.G7.** To examine the ability of various vaccines to activate CD4+ T cells, lymphocytes from mice immunized with IL18DC-E.G7, OVA257–264-pulsed DCs, DC-E.G7 conjugates, or PBS were restimulated in *vitro* with irradiated E.G7 cells. The amount of Th1 cytokines IL-2 and IFN-γ and Th2 cytokine IL-10 in the supernatants was then quantitated by the corresponding ELISA assays. IL-2, IFN-γ, and IL-10 were all induced in lymphocytes from mice immunized with OVA257–264-pulsed DCs, DC-E.G7 conjugates, or IL18DC-E.G7 conjugates (Fig. 4). Immunization with IL18DC-E.G7 led to the production of a significantly higher amount of IL-2 (1532 ± 69 pg/ml) and IFN-γ (2085 ± 298 pg/ml) than did immunization with OVA257–264-pulsed DCs (IL-2, 524 ± 75 pg/ml; IFN-γ, 420 ± 51 pg/ml) or DC-E.G7 (IL-2, 853 ± 103 pg/ml; IFN-γ, 997 ± 124 pg/ml; Fig. 4, A and B). Immunization with these vaccines also resulted in the production of IL-10 after restimulation with E.G7 (Fig. 4C). However, in contrast to secretion of Th1 cytokines, immunization with DC-E.G7 or IL18DC-E.G7 led to the production of a similar amount of IL-10 (DC-E.G7, 763 ± 97 pg/ml; IL18DC-E.G7, 699 ± 93 pg/ml) after *in vitro* restimulation with E.G7 (Fig. 4C). These data indicate that IL-18 enhances antitumor immune response by promoting generation of Th1 cells and support the notion that effective antitumor immune response requires Th cells.

**Antitumor Immunity Induced by IL18DC-E.G7 Vaccine in IFN-γ-depleted and IL-4+/- Mice.** To determine whether the effect of IL-18 is related to IFN-γ, we performed experiments in mice treated with anti-IFN-γ antibody. Without immunization, the growth of E.G7 tumors in normal C57BL/6 mice, control IgG-treated mice, and anti-

### Table 1 Tumor-free mice after immunotherapy with IL-18 gene-modified DC-E.G7 vaccine followed by rechallenge with wild-type E.G7 cells

<table>
<thead>
<tr>
<th>Groups</th>
<th>Tumor-free mice&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Tumor-free mice&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL18DC-E.G7 vaccine</td>
<td>9/10</td>
<td>5/5</td>
</tr>
<tr>
<td>LacZ.DC-E.G7 vaccine</td>
<td>5/10</td>
<td>2/5</td>
</tr>
<tr>
<td>DC-E.G7 vaccine</td>
<td>4/10</td>
<td>ND</td>
</tr>
<tr>
<td>DC/OVA257–264</td>
<td>6/10</td>
<td>3/5</td>
</tr>
<tr>
<td>IL18DC</td>
<td>2/10</td>
<td>ND</td>
</tr>
<tr>
<td>DC</td>
<td>1/10</td>
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<tr>
<td>PBS</td>
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<tr>
<td>Naive mice&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0/10</td>
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<sup>a</sup> The number of tumor-free mice 3 months after tumor inoculation.
<sup>b</sup> Five tumor-free mice in each group after IL-18 gene-modified DC-E.G7 vaccine were rechallenged with wild-type 10<sup>6</sup> E.G7 cells, and data represent the number of tumor-free mice after another 3 months.
<sup>c</sup> ND, no data.
<sup>d</sup> Naive mice received neither tumor inoculation nor immunotherapy. These mice were challenged with E.G7 cells as a control.

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Fig. 2. Tumor weights in mice after immunotherapy with IL-18 gene-modified DC-E.G7 vaccine. A, tumor-bearing mice (n = 10 in each group) were treated with IL18DC-E.G7 vaccine. LacZ gene-modified DC-E.G7 vaccine, DC-E.G7 vaccine, OVA257–264-pulsed DCs, IL-18 gene-modified DCs, DCs alone, or PBS. B, anti-CD4, anti-CD8, or anti-natural killer asciences containing 0.1 mg of McAb were injected i.p. into each mouse 4 days and 1 day before tumor inoculation, and another 3 injections of McAbs were performed at 2, 5, and 8 days after tumor inoculation.

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IFN-γ antibody-treated mice showed no significant differences (Fig. 5A). The growth of E.G7 tumors in wild-type C57BL/6 mice and syngeneic IL-4−/− mice was also similar at the time of examination (Fig. 5B). When the tumor-bearing mice were treated with anti-IFN-γ, the ability of the DC-E.G7 and IL18DC-E.G7 vaccines to inhibit tumor growth was significantly reduced. Moreover, there was no difference between the potency of the DC-E.G7 and IL18DC-E.G7 vaccines in anti-IFN-γ-treated mice. In contrast, the antitumor effects of DC-E.G7 and IL18DC-E.G7 vaccines were largely unaffected in IL-4−/− mice. Furthermore, immunization with IL18DC-E.G7 vaccine still induced stronger antitumor immunity than did that with DC-E.G7 in these IL-4−/− mice. These results demonstrated that IFN-γ is a critical mediator of antitumor immunity induced by the DC-E.G7 conjugates and is responsible for the stronger antitumor immunity induced by IL18DC-E.G7 conjugates.

DISCUSSION

DCs are the most potent antigen-presenting cells identified thus far and are crucial for priming the immune response. An increasing number of studies have demonstrated that immunization with DC-based vaccines is capable of inducing a specific CTL and antitumor immune response. For example, DCs pulsed with MHC class I-restricted peptide induced antigen-specific CTL-mediated protective tumor immunity in a number of experimental system and human trials (8, 29). However, the efficacy of peptide-pulsed DCs may be limited because the peptides could only stay bound to MHC molecules transiently due to low peptide binding affinity, dissociation of peptide-MHC complexes, and MHC turnover (30). To allow presentation of tumor antigen in a long term and stable manner, DCs were transduced with tumor antigen genes to elicit protective and therapeutic antitumor immunity (30, 31). These approaches require identifying tumor-specific antigens for individual tumors and demonstrating their recognition by CTLs, a process that is difficult and tedious. Therefore, antigens or cDNAs that can be used to pulse or transfect DCs for immunotherapy are very limited at the present time (9, 10). An interesting alternative is to treat DCs with unfractionated tumor cell lysates or even tumor-derived mRNA to induce effective antitumor immunity (11, 32, 33). Furthermore, DCs transfected with cDNA amplified from a small amount of tumor cells can induce CTLs and antitumor immunity, indicating that this strategy might be useful in various clinical situations in which tumor samples are limited. Another promising and efficient approach is to use the fusion of DCs and tumor cells as a vaccine (34–36). The success of this strategy depends on the generation and selection of hybrids that are stable and retain the critical components for stimulating the immune system, which might be quite time-consuming and challenging. It has been shown that a short-term coculture of DCs and tumor cells could result in rapid, efficient, and stable DC-tumor cell conjugates, which are an effective immunogen that can induce protective and therapeutic tumor rejection (27). In this study, we further demonstrated that DCs and E.G7 conjugates could induce tumor-specific CTLs, antigen-specific Th1 cytokine-producing cells, and antitumor immunity. In our system, DC-tumor conjugates consistently induced stronger antitumor immunity than DCs pulsed with peptides. Because we also observed a higher percentage of peptide-specific CD8+ T cells induced by the conjugates, the stronger antitumor immunity is likely due to better antigen presentation rather than recognition of multiple epitopes. These results indicated that formation of DC and tumor cell conjugates represents a novel strategy for cancer immunotherapy that is convenient, potent, and does not require identification of tumor antigen.

Traditionally, detection of antigen-specific T cells relied on methods such as limiting dilution, in vitro sensitization, and ELISPOT.
Whereas the ELISPOT has limited sensitivity, limiting dilution and in vitro sensitization require the in vitro expansion of T cells, which would take several days and could be affected by a number of factors. The development of the peptide-MHC tetramer makes it possible to rapidly and directly detect antigen-specific T cells (22). Initial studies demonstrated that tetramers are specific and are very sensitive in detecting virus-specific CTLs generated after virus infections and are a valuable tool in following antiviral immunity in the course of virus infection (37). These studies led to the appreciation that the magnitude of the virus-specific CTL response was much greater than previously thought. It has also been shown that tetramer can detect tumor-specific CD8+ T cells in tumor-infiltrated lymph nodes from patients with melanoma (38). These antigen-specific CD8+ T cells can then be sorted and, after in vitro expansion, used for adaptive transfer therapy of cancer (39). Another study found that tetramer could be used to detect CD8+ T cells specific for melanoma-associated antigens in the peripheral blood of patients with metastatic melanoma (40). Interestingly, although they accounted for 0.02–2% of circulating CD8+ T cells, these tetramer-positive cells appeared to be anergic in vivo, which may explain why these tumor-specific T cells failed to control tumor growth in these patients. In melanoma patients immunized with specific peptides, there were significant increases in antigen-specific CD8+ T cells as determined by staining with peptide/MHC tetramers (41). However, the increase of such T cells was not associated with enhanced rates of tumor regression. It is not clear whether these results reflected the presence of specific immunosuppression in cancer patients or the inability of tetramers to identify specific CTLs. In the present study, we are able to make specific tetramer and detect antigen-specific CD8+ T cells in mice immunized with various vaccines. The percentages of tetramer-positive cells correlate well with the potency of vaccines and the CTL activities as determined by cytotoxicity assay indicating that staining with tetramer is a specific, convenient, and reliable method for detection of CTLs in evaluating tumor vaccines.

Whereas CD8+ T cells are critical effectors in antitumor immune response, CD4+ T cells also play an important role in immunity against tumors (42, 43). The possible mechanisms for CD4+ T cells to promote antitumor CTLs include production of cytokines such as IFN-γ, modification of DCs, and maintenance of CD8+ T-cell numbers (44, 45). It appears that the relative importance of each mechanism varied in different experimental systems. We found that depletion of CD4+ T cells before immunization abolished the antitumor immune response induced by DC-E.G7 conjugates, whereas depletion of CD4+ T cells after immunization had only a moderate effect on antitumor immunity. Therefore, a major role of CD4+ T cells in this system is to help prime CTLs. We expected that the Th1 cytokine IL-18 might enhance the antitumor responses induced by the vaccine. The data presented here demonstrated that the IL-18 gene-modified DC-E.G7 vaccine elicited more potent antitumor responses manifested by increased antigen-specific Th1 cytokine production, a higher percentage of tumor-specific CD8+ T cells, and significantly slower tumor growth. It also indicated that it is possible to use DCs as a vector to deliver IL-18 to promote an antitumor immune response. Although IL-18 was initially identified as IFN-γ-inducing factor, it also has multiple biological activities, such as promoting the production of granulocyte macrophage colony-stimulating factor and IL-2 and activating natural killer cells and macrophages, that could...
contribute to its antitumor activity (46). In mice immunized with DC-EG7 conjugates, treatment with anti-IFN-γ antibodies significantly blocked antitumor immune responses, indicating that IFN-γ is a critical mediator for the vaccine. It also suggested that the more potent antitumor immunity induced by IL18DC-EG7 in the absence of anti-IFN-γ may be due to IL18-induced production of IFN-γ. Consistent with the notion, the antitumor effect of IL18DC-EG7 vaccine is also largely blocked by anti-IFN-γ antibodies.

ACKNOWLEDGMENTS

We thank Jun Sun, Minmin Yuan, and Zhenfang Fan for excellent technical assistance in cell culture, animal care, and cytotoxicity assay; Hongmei Song for protein purification; and Dr. Minghai Zhang and Rui Zhang for flow cytometry.

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Cancer Res 2001;61:3735-3740.

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