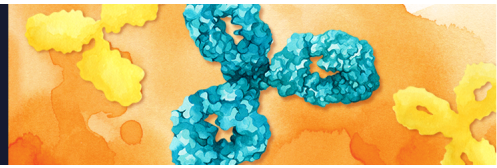


NEW InVivoSIM™
Biosimilar Antibodies
For Research Use Only

DISCOVER BioCell



 *The Journal of*
Immunology

Tumor-Derived TGF- β Reduces the Efficacy of Dendritic Cell/Tumor Fusion Vaccine

John Y. Kao, Yusong Gong, Chuan-Min Chen, Qiong-Duan Zheng and Jian-Jun Chen

This information is current as of June 15, 2021.

J Immunol 2003; 170:3806-3811; ;
doi: 10.4049/jimmunol.170.7.3806
<http://www.jimmunol.org/content/170/7/3806>

References This article **cites 47 articles**, 17 of which you can access for free at:
<http://www.jimmunol.org/content/170/7/3806.full#ref-list-1>

Why *The JI*? Submit online.

- **Rapid Reviews! 30 days*** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Fast Publication!** 4 weeks from acceptance to publication

**average*

Subscription Information about subscribing to *The Journal of Immunology* is online at:
<http://jimmunol.org/subscription>

Permissions Submit copyright permission requests at:
<http://www.aai.org/About/Publications/JI/copyright.html>

Email Alerts Receive free email-alerts when new articles cite this article. Sign up at:
<http://jimmunol.org/alerts>

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852
Copyright © 2003 by The American Association of
Immunologists All rights reserved.
Print ISSN: 0022-1767 Online ISSN: 1550-6606.



Tumor-Derived TGF- β Reduces the Efficacy of Dendritic Cell/Tumor Fusion Vaccine¹

John Y. Kao, Yusong Gong, Chuan-Min Chen, Qiong-Duan Zheng, and Jian-Jun Chen²

Dendritic cell (DC)-based antitumor vaccine is a novel cancer immunotherapy that is promising for reducing cancer-related mortality. However, results from early clinical trials were suboptimal. A possible explanation is that many tumors secrete immunosuppressive factors such as TGF- β , which may hamper host immune response to DC vaccine. In this study, we demonstrated that TGF- β produced by tumors significantly reduced the potency of DC/tumor fusion vaccines. TGF- β -secreting (CT26-TGF- β) stable mouse colon cancer cell lines were generated using a retroviral vector expressing TGF- β . A non-TGF- β -secreting (CT26-neo) cell line was generated using an empty retroviral vector. The efficacies of DC/tumor fusion vaccines were assessed *in vitro* and *in vivo*. DC/CT26-TGF- β fusion cells failed to induce a strong T cell proliferative response *in vitro*, mainly due to the effect of TGF- β on T cell responsiveness rather than DC stimulatory capability. Animals vaccinated with DC/CT26-TGF- β fusion vaccine had lower tumor-specific CTL activity and had significantly lower survival after tumor challenge as compared with animals immunized with DC/CT26-neo hybrids (45 vs 77%, $p < 0.05$). *Ex vivo* exposure of DCs to TGF- β did not appear to lessen the efficacy of DC vaccine. These data suggest that tumor-derived TGF- β reduces the efficacy of DC/tumor fusion vaccine via an *in vivo* mechanism. Neutralization of TGF- β produced by the fusion cells may enhance the effectiveness of DC-based immunotherapy. *The Journal of Immunology*, 2003, 170: 3806–3811.

The use of tumors as immunogens to induce antitumor immunity has become a major focus of cancer research. The dendritic cell (DC)³-based antitumor vaccine has emerged as a promising cancer immunotherapy with proven clinical efficacy (1–5). DCs are specialized APCs that can induce both the generation and proliferation of specific CTLs and Th cells through Ag presentation via MHC class I and class II molecules, respectively (6–10).

Considering the expected emergence of Ag-loss variants and the fact that most tumor Ags are not well-defined, DC-based vaccines often are generated using whole tumor-derived material (e.g., live tumors or tumor lysates). The fusion of tumor cells and DCs has been shown to induce strong immunologic responses (1, 11, 12), especially in animal studies; preliminary results from early clinical trials, however, have been disappointing. In one study, 60% of patients treated with DC/renal cell tumor fusion cells did not respond (1).

One disadvantage of using whole tumor substrate is that it may contain immunosuppressive factors that reduce vaccine efficacy. It has been documented that many tumor cell lines constitutively produce TGF- β , IL-10, or PGE-E₂ (13–17). *In vitro* studies have shown that these molecules inhibit NK cell- or lymphokine-activated killer cell-mediated cytotoxicity, or they inhibit IL-2 production (13, 18). Furthermore, TGF- β has been shown to inhibit CTL generation (19). The failure of the immune system to recog-

nize and destroy tumors may in part result from the suppression of immune function by these factors (20). Whether immunosuppressive factors secreted by tumors also paralyze the induction of antitumor immunity by DC vaccines, leading to treatment failure, is not known.

Previous studies of the effect of TGF- β on DCs have been inconclusive (21). Although one study showed that *in vitro* DC generation required TGF- β (22), other studies have shown that TGF- β can block DC maturation (23). Yet another study suggested that TGF- β reversibly regulates chemotaxis of DCs by regulating chemokine receptor expression (24). DCs infected with an adenoviral vector expressing TGF- β and IL-10 were found to induce partial immune tolerance; however, TGF- β alone did not (25). A recent study showed that TGF- β did not significantly impair the T cell stimulatory capacity of DCs *in vitro* (26).

To demonstrate the effect of TGF- β on DC antitumor vaccines, we generated a stable TGF- β -secreting tumor cell line using a retroviral vector encoding the constitutively active form of TGF- β . A non-TGF- β -secreting parental tumor cell line generated from the backbone vector was used as the control. Fusion vaccines generated separately using these two cell lines were evaluated *in vitro* and *in vivo*. We demonstrated that tumor-derived TGF- β reduced the ability of the fusion vaccine to stimulate T cell proliferation *in vitro*, inhibited the induction of the CTL response *in vivo* and weakened the protective ability of the DC vaccine against tumor challenge. To our knowledge, this is the first study to elucidate the inhibitory effect of tumor-derived immunosuppressive factors on DC/tumor fusion vaccines. These results suggest that blocking tumor-derived immunosuppressive factors may improve the clinical efficacy of DC vaccines.

Materials and Methods

Mice

Female BALB/c mice aged 6–8 wk were purchased from The Jackson Laboratory (Bar Harbor, ME) and housed at the Animal Maintenance Facility at the University of Michigan Health System (Ann Arbor, MI). Experiments were conducted at age 10–14 wk.

Division of Gastroenterology, Department of Internal Medicine, University of Michigan Medical Center, Ann Arbor, Michigan 48109

Received for publication July 23, 2002. Accepted for publication January 27, 2003.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ This study was partially supported by National Institutes of Health Grant CA87943-01 and Glaxo Institute of Digestive Health.

² Address correspondence and reprint requests to Dr. Jian-Jun Chen, Division of Gastroenterology, Department of Internal Medicine, University of Michigan Health System, 4510C Medical Science Research Building 1, 1150 West Medical Center Drive, Ann Arbor, Michigan 48109-0650. E-mail address: jjchen@umich.edu

³ Abbreviations used in this paper: DC, dendritic cell; CM, complete medium; PEG, polyethylene glycol.

Media and cytokines

Complete medium (CM) consisted of RPMI 1640 with 10% heat-inactivated FCS, 2 mM glutamine, 100 U/ml penicillin, and 100 μ g/ml streptomycin. CTL medium consisted of CM with 2-ME (Life Technologies, Grand Island, NY). Recombinant cytokines (R&D Systems, Minneapolis, MN) were diluted in CM: mouse GM-CSF (10 ng/ml), mouse IL-4 (10 ng/ml), human IL-2 (20 U/ml).

Tumor cell lines

CT26, a mouse colon adenocarcinoma cell line (27), and Renca, a mouse renal epithelial adenocarcinoma cell line (27), both grown in BALB/c mice, were propagated in CM.

Generation of a TGF- β -expressing CT26 cell line

A CT26 cell line stably transfected to express a constitutively active form of TGF- β 1 (CT26-TGF- β) was generated using a retrovirus that expressed TGF- β and a neomycin-resistant gene, kindly provided by Dr. G. Nabel (Vaccine Research Center, National Institutes of Health, Bethesda, MD) (28). A control CT26 cell line (CT26-neo) was generated similarly using the retrovirus backbone containing the neomycin-resistant gene alone.

Generation of bone marrow-derived DCs

Erythrocyte-depleted murine bone marrow cells were cultured in CM with 10 ng/ml GM-CSF and 10 ng/ml IL-4 at 1×10^6 cells/ml, as described (29). On day 6, nonadherent DCs were harvested by forceful pipetting and enriched by metrizamide centrifugation gradients (14.5% by weight) (Sigma-Aldrich, St. Louis, MO). The low density interface containing the DCs was collected by gentle pipette aspiration. The recovered DCs were washed twice with RPMI 1640 and cultured in CM with GM-CSF and IL-4.

DC/tumor fusion cell preparation

Bone marrow-derived DCs were fused with tumor cells at a DC-tumor ratio of 2:1 using 50% polyethylene glycol (PEG; m.w. 1450)/DMSO solution (Sigma-Aldrich), as described (28). Briefly, the DC/tumor cell suspension was washed twice with RPMI 1640 prewarmed to 37°C. PEG (50%, 1 ml) was added over 1 min and the suspension was stirred gently for 1–2 min. Prewarmed RPMI 1640 (1 ml) was then added over 1 min and the suspension was stirred. An additional 3 ml of RPMI 1640 was added over 3 min, after which 10 ml of RPMI 1640 was added slowly. After a 5-min incubation at 37°C, the resultant cell mixture was pelleted and grown overnight in CM with GM-CSF and IL-4.

Determination of fusion efficiency

DCs were incubated with Mouse Fc Block (BD PharMingen, San Diego, CA) 1 μ g/100 ml for 20 min at 4°C followed by labeling with R-PE-conjugated anti-mouse CD11c (BD PharMingen) or PE-conjugated IgG isotype control (BD PharMingen). Tumor cells (CT26-neo or CT26-TGF- β) were labeled with a PKH67-Green Fluorescent Cell Linker kit (Sigma-Aldrich) (30). DCs and tumor cells were then fused with 50% PEG as described above. The fusion efficiency was determined by counting the percent of double-stained cells using Coulter Epics XL Cytometer (Hialeah, FL). The number of intact fused cells following fusion was assessed by the trypan-blue exclusion test.

TGF- β production by fusion cells

DC/tumor fusion cells were sorted using Coulter Elite ESP Sorter (Coulter) and cultured at 1.5×10^5 cells/ml for 48 h in CM supplemented with IL-4 and GM-CSF. Bioactive TGF- β secretion was determined by ELISA without acidification of the samples.

Tumor lysate-pulsed DC preparation

DCs were pulsed with freeze-thawed tumor lysates at a DC-tumor ratio of 1:3, as described (31). CT26-TGF- β pulsed DCs and CT26-neo pulsed DCs were harvested and washed two times after an 18-h incubation.

Induction of proliferative T cell responses in vitro

Irradiated (16,000 rad) DC/tumor fusion cells were cocultured with naive syngeneic BALB/c splenocytes (1×10^4 cells) for 48 h. Alternatively, before fusion with DCs, tumors were pretreated with mitomycin C (Sigma-Aldrich) instead of radiation. Tritiated deoxythymidine (1 μ Ci/well; Amersham Biosciences, Piscataway, NJ) was added to each microtiter well and the plates were incubated for 24 h. At completion, the plates were harvested and the radioactivity was measured using a scintillation counter. Responses were reported as mean cpm \pm SEM from duplicate samples.

Animal studies

BALB/c mice were vaccinated twice (i.p.) on days 0 and 14 with DC/CT26-TGF- β fusion cells, DC/CT26-neo fusion cells, CT26-TGF- β -pulsed DCs, or CT26-neo-pulsed DCs (5×10^5 cells per injection). To test the efficacy of the vaccines, the animals were challenged with a lethal dose of CT26 (2×10^6 cells) on day 21. Animals were checked for tumors twice weekly, and tumor growth and survival times were recorded. Tumors were measured with a pair of Vernier calipers twice weekly and size was calculated according to the following equation: volume = $b^2 \times a/2$ where a is the longer diameter of the tumor and b the shorter diameter.

CTL assays

T cells enriched by nylon wool column separation were tested for cytolytic activity in a standard 6-h 51 Cr-release assay. CTLs (15×10^6 cells) were stimulated with irradiated tumor (12,000 rad) at a T cell-tumor ratio of 20:1 in CTL medium for 5 days. IL-2 (20 U/ml) was added on day 2. Target cells (1×10^6 cells) were incubated at 37°C with 51 Cr (50 μ Ci) (Amersham Biosciences) in 100 μ l of CTL medium for 1 to 2 h, washed twice with RPMI 1640, and resuspended in CTL medium at 1×10^5 cells/ml. Target cells labeled with 51 Cr (1×10^4 cells/100 μ l) were placed in each well of 96-well plates, and 100 μ l of effector T cells for each dilution was added. Plates were incubated for 6 h at 37°C. The supernatant from each well was harvested and the amount of 51 Cr radioactivity released was measured in a gamma counter. The percentage of specific lysis was calculated.

Serum TGF- β concentration in animals that received a single immunization

Animals were anesthetized and whole blood was collected from tail veins 6 and 14 days after a single immunization with 5×10^5 DC/CT26-TGF- β fusion cells or DC/CT26-neo fusion cells. Time points earlier than 6 days (day 1 and 3) were obtained in a separate experiment. Serum samples were acidified with 1 M HCl (pH 2.0) as described (32), incubated at 4°C for 1 h, and neutralized with 1 M NaOH (pH 7.0) shortly before use. TGF- β concentration in each sample was determined using a standard ELISA (R&D Systems).

Statistical analysis

Unpaired Student's *t* test analyses were performed using GraphPad Prism version 3.00 for Windows (GraphPad Software, San Diego CA).

Results

Generation of TGF- β -secreting tumor cell line

To demonstrate the effect of TGF- β on the DC-tumor fusion vaccine, CT26 tumor cells were genetically modified to overexpress TGF- β . The successful generation of CT26-TGF- β was confirmed by detecting the TGF- β level in the tumor cell supernatant (Fig. 1). CT26-TGF- β cells secreted 10 times more TGF- β than CT26-neo cells (11 ng/ml vs 0.4 ng/ml). The enhanced TGF- β expression of CT26-TGF- β is comparable to some murine and human cancer cell lines known to spontaneously secrete high TGF- β levels (20, 33).

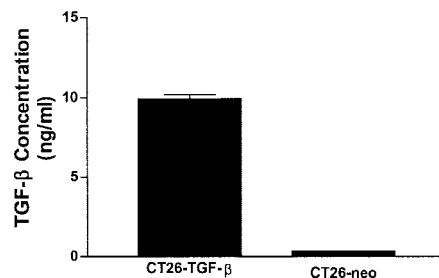


FIGURE 1. TGF- β expression of CT26-TGF- β and CT26-neo cells. Tumor cells, CT26-TGF- β or CT26-neo, were seeded at 5×10^5 cells/ml in a 6-well plate and cultured for 48 h in CM. Supernatants were collected and acidified as described in *Materials and Methods*. TGF- β concentration in each supernatant was determined by ELISA. Results are representative of three independent experiments.

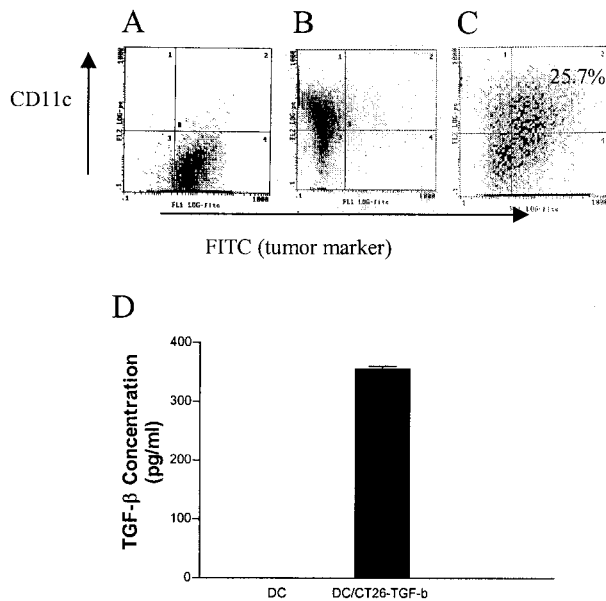


FIGURE 2. Determination of fusion efficiency and TGF- β secretion by DC/tumor fusion cells. *A*, FACS analysis showing CT26-TGF- β tumor cells labeled with PKH67-green fluorescent dye before PEG treatment. *B*, DCs labeled with PE-conjugated anti-CD11c before fusion. *C*, PEG effectively fused DC and CT26-TGF- β fusion cells. The fusion efficiency was determined by the percent of double-stained cells (25.7%). *D*, DC/CT26-TGF- β fusion cells secrete bioactive TGF- β . Fusion cells were sorted by a FACS sorter and 1.5×10^5 fusion cells/ml were cultured for 48 h. ELISA was performed on nonacidified supernatants to detect tumor-derived TGF- β . The supernatant from DC culture (1.5×10^5 cells/ml) was included as the control.

DC vaccine derived from TGF- β -secreting tumor continue to secrete TGF- β

To generate DC/tumor fusion vaccines, CT26-neo or CT26-TGF- β were fused with DC cells as described in *Materials and Methods*. To determine the fusion efficiency, DCs were stained with PE-conjugated anti-mouse CD11c and tumor cells were labeled with a PKH67-Green Fluorescent Cell Linker kit as previously described (30). The fusion efficiency was determined by the percent of double-stained cells using FACS analysis (Fig. 2). The mean fusion efficiency was 21.3% (range 17–25.3%). This is consistent with the reported fusion efficiencies of 12.5–30% (34–36). We did not observe significant variation in the fusion efficiency between DC/CT26-neo and DC/CT26-TGF- β . Approximately 50% of the fused cells were viable per trypan blue exclusion test (data not shown). To confirm that DC/CT26-TGF- β fusion cells continued to secrete bioactive TGF- β , flow sorted DC/CT26-TGF- β fusion cells were cultured for 48 h and the supernatants were analyzed by ELISA. Because CT26-TGF- β tumor cells constitutively secrete the active form of TGF- β , the supernatants were not acidified; therefore, only tumor-derived TGF- β was detected and not inactive TGF- β produced by DCs. The supernatant from a cell culture containing only DCs served as the control. The concentrations of bioactive TGF- β were 356 ± 5.35 pg/ml for sorted DC/CT26-TGF- β fusion cells and were undetectable for DC alone (Fig. 2D). These results clearly demonstrated that DC/CT26-TGF- β fusion cells continue to secrete TGF- β .

DC vaccine derived from TGF- β -secreting tumor is less effective in inducing T cell proliferation in vitro

The inhibitory effect of tumor-derived TGF- β on the DC vaccine was first demonstrated in vitro. We investigated the capability of

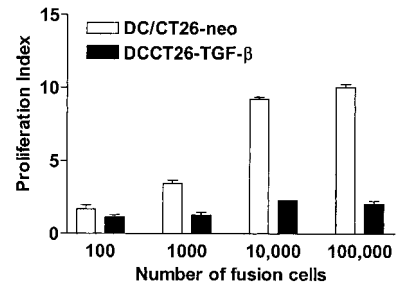


FIGURE 3. DC/CT26-TGF- β fusion cells failed to induce a strong T cell proliferation response in vitro. Naive T cells (2×10^5 cells/well) were cocultured with different numbers of irradiated DC/tumor fusion cells (DC/CT26-neo or DC/CT26-TGF- β) and incubated for 48 h. T cell proliferation was determined by [3 H]thymidine assay and expressed as proliferation index, defined as the ratio of the measured radioactivity in wells with T cells stimulated with DC/tumor fusion cells over that of T cell alone. No significant proliferative activity was detected in T cells alone or fusion cells alone (data not shown). Results are representative of two independent experiments.

DC/CT26-TGF- β fusion cells and DC/CT26-neo fusion cells to prime naive syngeneic T cells by measuring the T cell proliferative response. Stimulation of naive syngeneic BALB/c T cells with DC/CT26-TGF- β fusion cells failed to induce a significant proliferative response (Fig. 3). The failure of T cells to respond to DC/TGF- β fusion cells in vitro suggests that tumor-derived TGF- β may either reduce the ability of DCs to present Ag to T cells or directly suppress T cell proliferation without affecting DC.

Exposure of DCs to TGF- β ex vivo did not affect DC T cell stimulatory capacity

To determine whether tumor-secreted TGF- β affects the functional capacity of DCs, we cultured DC/CT26-neo fusion cells overnight with supernatants from DC/CT26-TGF- β fusion cells and assessed the ability of the fusion cells to stimulate T cells. Overnight ex vivo exposure of DC/CT26-neo fusion cells to TGF- β did not reduce the ability of fusion cells to stimulate T cell proliferation (Fig. 4). This finding is consistent with the report that exposure to TGF- β does not inhibit the ability of DCs to present Ag in vitro (26).

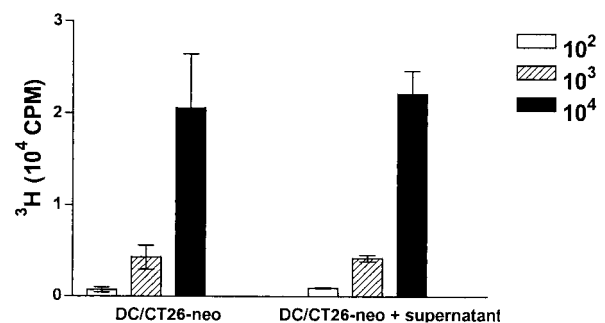


FIGURE 4. Stimulatory effect of DC/CT26-neo fusion cells was unaffected by overnight culture in supernatant of DC/CT26-TGF- β . DC/CT26-neo fusion cells were cultured in an overnight supernatant of DC/CT26-TGF- β fusion cell culture. T cell proliferation assays were performed using [3 H]thymidine and proliferative activity was expressed as cpm. *Left panel*, Three dilutions of DC/CT26-neo fusion cells (10^2 , 10^3 , and 10^4) were cocultured with naive T cells (10^4 cells). *Right panel*, Similarly, DC/CT26-neo fusion cells incubated overnight with supernatant containing tumor-derived TGF- β were cocultured with naive T cells. Results are representative of two independent experiments.

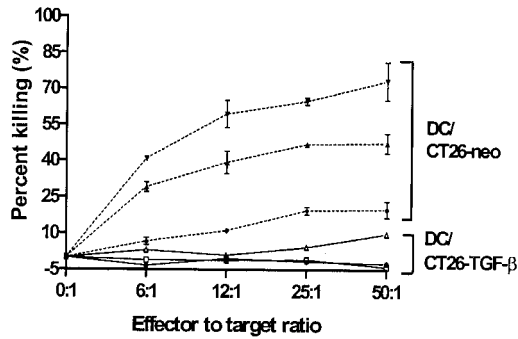


FIGURE 5. Induction of CTL response in vivo was reduced with DC/CT26-TGF-β fusion vaccine. BALB/c mice ($n = 3$) were immunized once on day 0 with 5×10^5 DC/CT26-neo or DC/CT26-TGF-β fusion cells. T cells obtained from the spleen on day 14 were stimulated with irradiated CT26 for 5 days. ^{51}Cr -labeled CT26 cells were cocultured with stimulated CTL for 6 h at the indicated E:T ratios. CTL activity was expressed as the percent of cells killed. Each line on the graph represents an individual mouse.

DC vaccine derived from TGF-β-secreting tumor is less effective in inducing CTL activity in vivo

To determine the in vivo effect of tumor-derived TGF-β on DC vaccine, animals were immunized with a single dose of DC/CT26-neo fusion cells or DC/CT26-TGF-β fusion cells. The ability of the DC/tumor fusion vaccine to induce tumor-specific CTL activities was assessed by CTL assay. All three mice vaccinated with DC/CT26-neo fusion cells developed a stronger CTL response compared with the response in three of three mice vaccinated with DC/CT26-TGF-β fusion cells (Fig. 5).

DCs fused with TGF-β-secreting tumor cells are less effective in protecting mice from tumor challenge

The protective immunity induced by DC/tumor fusion cells was assessed in vivo by challenging mice with a lethal dose of CT26 after immunization. BALB/c mice were vaccinated with DC/CT26-TGF-β fusion cells or DC/CT26-neo fusion cells (5×10^5 cells) on days 0 and 14. An inoculation of CT26 (2×10^6 cells, s.c.) was administered on day 21. Higher tumor incidence and lower survival rates occurred in mice immunized with DC/CT26-TGF-β fusion cells, compared with those immunized with DC/

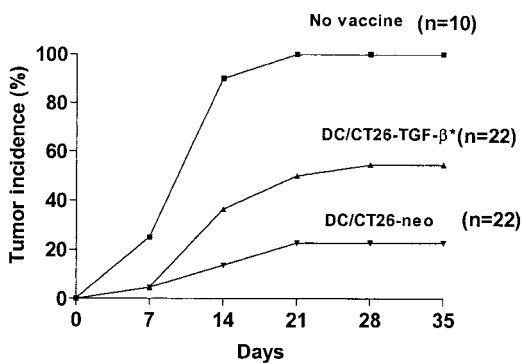


FIGURE 6. Higher tumor incidence in mice immunized with DC/CT26-TGF-β fusion vaccine. Mice ($n = 22$) were immunized twice with DC/CT26-TGF-β or DC/CT26-neo fusion cells on days 0 and 14, and inoculated with CT26 (2×10^6 cells) on day 21. Tumor development was monitored and recorded as positive growth if tumor size was >5 mm in its largest diameter. Tumor incidence was significantly higher in the DC/CT26-TGF-β group as compared with the DC/CT26-neo group (*, $p < 0.05$).

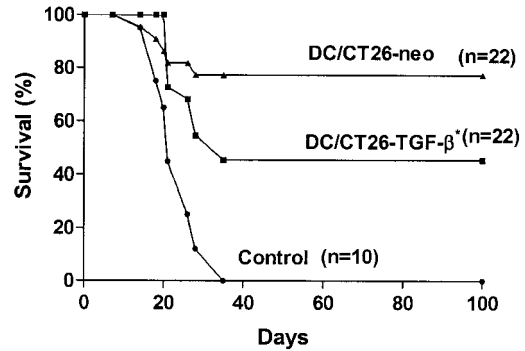


FIGURE 7. Lower survival rates in mice immunized with DC/CT26-TGF-β fusion vaccine. BALB/c mice received two immunizations of either DC/CT26-neo fusion cells ($n = 22$) or DC/CT26-TGF-β fusion cells ($n = 22$), as described in Fig. 6, or mice ($n = 10$) were not vaccinated. Animals were sacrificed once tumor size reached 15 mm in its largest diameter. Survival at 100 days after tumor challenge was significantly lower in the DC/CT26-TGF-β group compared with the DC/CT26-neo group (*, $p < 0.05$).

CT26-neo fusion cells, $p < 0.05$ (Figs. 6 and 7, and Table I). All surviving animals remained tumor-free beyond 1 year.

Decreased efficacy of DC/CT26-TGF-β fusion cell vaccine is not due to higher serum TGF-β level

To better understand how tumor-derived TGF-β reduces the ability of DC/CT26-TGF-β fusion cells to protect against tumor challenge, we compared the serum TGF-β level of mice immunized with DC/CT26-TGF-β fusion cells to that of mice immunized with DC/CT26-neo fusion cells. A single vaccination was administered on day 0 and serum TGF-β levels were compared on days 1, 3, 6, and 14. No significant difference in the total serum TGF-β level was detected between the two groups for all four time points (Fig. 8 and data not shown). Because CT26-TGF-β tumor cells constitutively secrete the active form of TGF-β, further tests of non-acidified serum samples for the active form of TGF-β were performed. We found no detectable levels of tumor-derived TGF-β (data not shown). These results suggest that TGF-β secreted from DC/CT26-TGF-β fusion cells probably exerts its effect locally rather than systemically.

Exposure of DCs to TGF-β ex vivo did not affect DC vaccine efficacy

The ability to generate tumor lysate-pulsed DC vaccines offers an opportunity to study the consequence of ex vivo TGF-β exposure on the efficacy of DC vaccine. DCs were pulsed with the tumor lysate of either CT26-TGF-neo or CT26-TGF-β overnight. The cells from each group were then washed to remove tumor-derived TGF-β and used to immunize mice (two doses separated by 14

Table I. Survival of BALB/c mice after tumor challenge

Vaccine	Survival
No Vaccine	0/10 (0%)
DC/CT26-TGF-β fusion cells	10/22 (45.5%) ^a
DC/CT26-neo fusion cells	17/22 (77.3%)
DC + CT26-TGF-β lysate	8/17 (47.1%) ^b
DC + CT26-neo lysate	7/17 (41.2%)

^a $p < 0.05$ compared to DC/CT26-neo fusion cells.

^b $p > 0.05$ compared to DC + CT26-neo lysate.

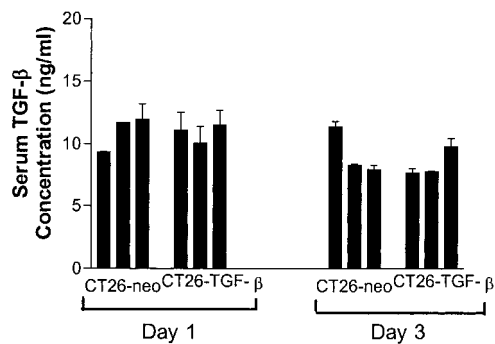


FIGURE 8. Serum TGF- β levels in mice immunized with DC/tumor fusion vaccine. BALB/c mice ($n = 3$ in each group) were immunized once with DC/CT26-neo fusion cells or DC/CT26-TGF- β fusion cells as described in Fig. 5. Sera collected on days 1 and 3 were analyzed by ELISA to determine TGF- β levels. No significant difference was found between the two groups.

days). Seven days after the second vaccination, animals were challenged with a lethal dose of CT26. The posttumor challenge survival rates between the two groups were similar, suggesting that overnight exposure of DCs to TGF- β ex vivo did not affect the ability of DCs to induce tumor-rejecting CTL activities (Table I). Interestingly, our results, although not statistically significant, also revealed that the DC/CT26-neo fusion vaccine tends to be more effective than the CT26-neo lysate-pulsed DC vaccine in preventing tumor growth (77.3 vs 41.2; $p > 0.05$).

Discussion

The potential of DC-based vaccines to eradicate unresectable tumors has stimulated intense investigation. Most studies have focused on improving vaccine efficacy using adjunct treatment or the expression of cytokines and costimulatory molecules (37–46). Little is known about the impact of tumor-derived immunosuppressive factors on the efficacy of the DC vaccine. Considering that many tumors express immunosuppressive factors and that DC/tumor fusion vaccines are being used in clinical settings, we addressed the question of whether tumor-derived immunosuppressive factors could limit the efficacy of the DC vaccine. We observed that the tumor-specific CTL response induced by DCs fused with TGF- β -expressing tumor was decreased both in vitro and in vivo. Furthermore, protection against tumor challenge was significantly compromised suggesting that tumor-derived immunosuppressive factors can significantly reduce the efficacy of the DC/tumor fusion vaccine.

The findings that TGF- β in tumor lysate did not significantly affect DC vaccine efficacy suggest that overnight exposure of DCs to TGF- β in vitro does not reduce DC Ag-presenting capability. This was further supported by the finding that culturing DC/CT26-neo fusion cells with supernatant from the DC/CT26-TGF- β fusion cell culture did not affect the ability of DC/CT26-neo fusion cells to simulate T cell proliferation in vitro (Fig. 4). Thus, the most likely mechanism for the reduced efficacy of the DC/CT26-TGF- β fusion vaccine is the in vivo effect of TGF- β on the responsiveness of T cells to DC priming. Failure to detect increased TGF- β levels in the sera of mice immunized with the DC/CT26-TGF- β fusion vaccine further suggests that the amount of TGF- β in the microenvironment of lymphoid organs where DCs interact with lymphocytes may be critical. These results are consistent with the finding that transgenic mice that express a dominant-negative TGF- β receptor on T cells are capable of rejecting tumor challenge (47). Therefore, strategies to neutralize the tumor-derived TGF- β

secreted by the DC/tumor fusion vaccine may further enhance the cellular immunity of DC-primed T cells to eradicate tumors.

Because TGF- β not only suppresses immunity but also inhibits tumor proliferation, blocking TGF- β systemically may lead to accelerated tumor growth. Neutralization of TGF- β in the microenvironment of lymphoid organs may reverse immune suppression without blocking the inhibitory effect of TGF- β on tumorigenesis. This can be achieved by genetically engineering DCs to express a secreted form of the dominant-negative TGF- β receptor, blocking not only tumor-derived TGF- β , but also host-derived TGF- β in the microenvironment.

In summary, our study demonstrates that tumor-derived immunosuppressive factors can significantly reduce the efficacy of DC-based tumor vaccines. Our results also suggest that, in addition to the use of immunostimulatory factors, the blocking of tumor-derived immunosuppressive molecules may optimize the effectiveness of DC vaccines in tumor therapy. These results have important clinical implications for DC-based cancer therapy.

Acknowledgments

We thank Gwo-Hsiao Chen and Chris Kirk for technical support of the DC isolation techniques.

References

- Kugler, A., G. Stuhler, P. Walden, G. Zoller, A. Zobywalski, P. Brossart, U. Trefzer, S. Ullrich, C. A. Muller, V. Becker, et al. 2000. Regression of human metastatic renal cell carcinoma after vaccination with tumor cell-dendritic cell hybrids. *Nat. Med.* 6:332.
- Tjoa, B. A., S. J. Simmons, A. Elgamal, M. Rogers, H. Ragde, G. M. Kenny, M. J. Troychak, A. L. Boynton, and G. P. Murphy. 1999. Follow-up evaluation of a phase II prostate cancer vaccine trial. *Prostate* 40:125.
- Murphy, G. P., B. A. Tjoa, S. J. Simmons, H. Ragde, M. Rogers, A. Elgamal, G. M. Kenny, M. J. Troychak, M. L. Salgaller, and A. L. Boynton. 1999. Phase II prostate cancer vaccine trial: report of a study involving 37 patients with disease recurrence following primary treatment. *Prostate* 39:54.
- Nestle, F. O., S. Aljagic, M. Gilliet, Y. Sun, S. Grabbe, R. Dummer, G. Burg, and D. Schadendorf. 1998. Vaccination of melanoma patients with peptide- or tumor lysate-pulsed dendritic cells. *Nat. Med.* 4:328.
- Hsu, F. J., C. Benike, F. Fagnoni, T. M. Liles, D. Czerwinski, B. Taidi, E. G. Engleman, and R. Levy. 1996. Vaccination of patients with B-cell lymphoma using autologous antigen-pulsed dendritic cells. *Nat. Med.* 2:52.
- Ohshima, Y., and G. Delespesse. 1997. T cell-derived IL-4 and dendritic cell-derived IL-12 regulate the lymphokine-producing phenotype of alloantigen-primed naive human CD4 T cells. *J. Immunol.* 158:629.
- Brossart, P., A. W. Goldrath, E. A. Butz, S. Martin, and M. J. Bevan. 1997. Virus-mediated delivery of antigenic epitopes into dendritic cells as a means to induce CTL. *J. Immunol.* 158:3270.
- Shen, Z., G. Reznikoff, G. Dranoff, and K. L. Rock. 1997. Cloned dendritic cells can present exogenous antigens on both MHC class I and class II molecules. *J. Immunol.* 158:2723.
- Nair, S., F. Zhou, R. Reddy, L. Huang, and B. T. Rouse. 1992. Soluble proteins delivered to dendritic cells via pH-sensitive liposomes induce primary cytotoxic T lymphocyte responses in vitro. *J. Exp. Med.* 175:609.
- Porgador, A., and E. Gilboa. 1995. Bone marrow-generated dendritic cells pulsed with a class I-restricted peptide are potent inducers of cytotoxic T lymphocytes. *J. Exp. Med.* 182:255.
- Walden, P. 2000. Hybrid cell vaccination for cancer immunotherapy. *Adv. Exp. Med. Biol.* 465:347.
- Gong, J., V. Apostolopoulos, D. Chen, H. Chen, S. Koido, S. J. Gendler, I. F. McKenzie, and D. Kufe. 2000. Selection and characterization of MUC1-specific CD8⁺ T cells from MUC1 transgenic mice immunized with dendritic-carcinoma fusion cells. *Immunology* 101:316.
- Kuppner, M. C., Y. Sawamura, M. F. Hamou, and N. de Tribolet. 1990. Influence of PGE₂- and cAMP-modulating agents on human glioblastoma cell killing by interleukin-2-activated lymphocytes. *J. Neurosurg.* 72:619.
- Torre-Amione, G., R. D. Beauchamp, H. Koeppen, B. H. Park, H. Schreiber, H. L. Moses, and D. A. Rowley. 1990. A highly immunogenic tumor transfected with a murine transforming growth factor type β 1 cDNA escapes immune surveillance. *Proc. Natl. Acad. Sci. USA* 87:1486.
- Maxwell, M., T. Galanopoulos, J. Neville-Golden, and H. N. Antoniades. 1992. Effect of the expression of transforming growth factor- β 2 in primary human glioblastomas on immunosuppression and loss of immune surveillance. *J. Neurosurg.* 76:799.
- Bomstein, Y., R. Ophir, H. Harshemes, and S. Ben-Efraim. 1993. Release of immunosuppressive factor(s) by MOPC-315 murine plasmacytoma cells: a possible mechanism of defence. *Anticancer Res.* 13:2125.
- Daughaday, W. H., and T. F. Deuel. 1991. Tumor secretion of growth factors. *Endocrinol. Metab. Clin. North Am.* 20:539.

18. Rook, A. H., J. H. Kehrl, L. M. Wakefield, A. B. Roberts, M. B. Sporn, D. B. Burlington, H. C. Lane, and A. S. Fauci. 1986. Effects of transforming growth factor β on the functions of natural killer cells: depressed cytolytic activity and blunting of interferon responsiveness. *J. Immunol.* 136:3916.
19. Rich, S., M. Seelig, H. M. Lee, and J. Lin. 1995. Transforming growth factor β costimulated growth and regulatory function of staphylococcal enterotoxin B-responsive CD8⁺ T cells. *J. Immunol.* 155:609.
20. Hsieh, C. L., D. S. Chen, and L. H. Hwang. 2000. Tumor-induced immunosuppression: a barrier to immunotherapy of large tumors by cytokine-secreting tumor vaccine. *Hum. Gene Ther.* 11:681.
21. Strobl, H., and W. Knapp. 1999. TGF- β 1 regulation of dendritic cells. *Microbes Infect.* 1:1283.
22. Riedl, E., H. Strobl, O. Majdic, and W. Knapp. 1997. TGF- β 1 promotes in vitro generation of dendritic cells by protecting progenitor cells from apoptosis. *J. Immunol.* 158:1591.
23. Yamaguchi, Y., H. Tsumura, M. Miwa, and K. Inaba. 1997. Contrasting effects of TGF- β 1 and TNF- α on the development of dendritic cells from progenitors in mouse bone marrow. *Stem Cells* 15:144.
24. Sato, K., H. Kawasaki, H. Nagayama, M. Enomoto, C. Morimoto, K. Tadokoro, T. Juji, and T. A. Takahashi. 2000. TGF- β 1 reciprocally controls chemotaxis of human peripheral blood monocyte-derived dendritic cells via chemokine receptors. *J. Immunol.* 164:2285.
25. Gray, J. D., M. Hirokawa, and D. A. Horwitz. 1994. The role of transforming growth factor β in the generation of suppression: an interaction between CD8⁺ T and NK cells. *J. Exp. Med.* 180:1937.
26. Lin, C. M., F. H. Wang, and P. K. Lee. 2002. Activated human CD4⁺ T cells induced by dendritic cell stimulation are most sensitive to transforming growth factor- β : implications for dendritic cell immunization against cancer. *Clin. Immunol.* 102:96.
27. Fearon, E. R., and B. Vogelstein. 1990. A genetic model for colorectal tumorigenesis. *Cell* 61:759.
28. Wang, J., S. Saffold, X. Cao, J. Krauss, and W. Chen. 1998. Eliciting T cell immunity against poorly immunogenic tumors by immunization with dendritic cell-tumor fusion vaccines. *J. Immunol.* 161:5516.
29. Fields, R. C., J. J. Osterholzer, J. A. Fuller, E. K. Thomas, P. J. Geraghty, and J. J. Mule. 1998. Comparative analysis of murine dendritic cells derived from spleen and bone marrow. *J. Immunother.* 21:323.
30. Holmes, L. M., J. Li, R. P. Sticca, T. E. Wagner, and Y. Wei. 2001. A rapid, novel strategy to induce tumor cell-specific cytotoxic T lymphocyte responses using instant dendritomas. *J. Immunother.* 24:122.
31. Cohen, P. J., P. A. Cohen, S. A. Rosenberg, S. I. Katz, and J. J. Mule. 1994. Murine epidermal Langerhans cells and splenic dendritic cells present tumor-associated antigens to primed T cells. *Eur. J. Immunol.* 24:315.
32. Tzai, T. S., A. L. Shiau, C. S. Lin, C. L. Wu, and J. S. Lin. 1997. Modulation of the immunostimulating effect of autologous tumor vaccine by anti-TGF- β antibody and interferon- α on murine MBT-2 bladder cancer. *Anticancer Res.* 17:1073.
33. Fischer, J. R., H. Darjes, H. Lahm, M. Schindel, P. Drings, and P. H. Krammer. 1994. Constitutive secretion of bioactive transforming growth factor β 1 by small cell lung cancer cell lines. *Eur. J. Cancer* 30A:2125.
34. Li, J., L. M. Holmes, K. J. Franek, K. E. Burgin, T. E. Wagner, and Y. Wei. 2001. Purified hybrid cells from dendritic cell and tumor cell fusions are superior activators of antitumor immunity. *Cancer Immunol. Immunother.* 50:456.
35. Lindner, M., and V. Schirrmacher. 2002. Tumour cell-dendritic cell fusion for cancer immunotherapy: comparison of therapeutic efficiency of polyethyleneglycol versus electro-fusion protocols. *Eur. J. Clin. Invest.* 32:207.
36. Homma, S., G. Toda, J. Gong, D. Kufe, and T. Ohno. 2001. Preventive antitumor activity against hepatocellular carcinoma (HCC) induced by immunization with fusions of dendritic cells and HCC cells in mice. *J. Gastroenterol.* 36:764.
37. Wan, Y., J. Bramson, A. Pilon, Q. Zhu, and J. Gauldie. 2000. Genetically modified dendritic cells prime autoreactive T cells through a pathway independent of CD40L and interleukin 12: implications for cancer vaccines. *Cancer Res.* 60:3247.
38. Chen, Y., P. Emtage, Q. Zhu, R. Foley, W. Muller, M. Hitt, J. Gauldie, and Y. Wan. 2001. Induction of ErbB-2/neu-specific protective and therapeutic antitumor immunity using genetically modified dendritic cells: enhanced efficacy by cotransduction of gene encoding IL-12. *Gene Ther.* 8:316.
39. Hellstrom, I., J. A. Ledbetter, N. Scholler, Y. Yang, Z. Ye, G. Goodman, J. Pullman, M. Hayden-Ledbetter, and K. E. Hellstrom. 2001. CD3-mediated activation of tumor-reactive lymphocytes from patients with advanced cancer. *Proc. Natl. Acad. Sci. USA* 98:6783.
40. Furumoto, K., S. Arai, S. Yamasaki, M. Mizumoto, A. Mori, N. Inoue, N. Isobe, and M. Imamura. 2000. Spleen-derived dendritic cells engineered to enhance interleukin-12 production elicit therapeutic antitumor immune responses. *Int. J. Cancer* 87:665.
41. Ju, D. W., Q. Tao, G. Lou, M. Bai, L. He, Y. Yang, and X. Cao. 2001. Interleukin 18 transfection enhances antitumor immunity induced by dendritic cell-tumor cell conjugates. *Cancer Res.* 61:3735.
42. Kikuchi, T., N. Miyazawa, M. A. Moore, and R. G. Crystal. 2000. Tumor regression induced by intratumor administration of adenovirus vector expressing CD40 ligand and naive dendritic cells. *Cancer Res.* 60:6391.
43. Kikuchi, T., S. Worgall, R. Singh, M. A. Moore, and R. G. Crystal. 2000. Dendritic cells genetically modified to express CD40 ligand and pulsed with antigen can initiate antigen-specific humoral immunity independent of CD4⁺ T cells. *Nat. Med.* 6:1154.
44. Kikuchi, T., M. A. Moore, and R. G. Crystal. 2000. Dendritic cells modified to express CD40 ligand elicit therapeutic immunity against preexisting murine tumors. *Blood* 96:91.
45. Shimizu, K., R. C. Fields, B. G. Redman, M. Giedlin, and J. J. Mule. 2000. Potentiation of immunologic responsiveness to dendritic cell-based tumor vaccines by recombinant interleukin-2. *Cancer J. Sci. Am.* 6(Suppl. 1):S67.
46. Zhang, W., X. Cao, and X. Huang. 1997. In vivo induction of antitumor immune response by tumor cells fused with GM-CSF gene-modified dendritic cells. *Zhonghua Yi Xue Za Zhi* 77:39.
47. Gorelik, L., and R. A. Flavell. 2001. Immune-mediated eradication of tumors through the blockade of transforming growth factor- β signaling in T cells. *Nat. Med.* 7:1118.