

Application of Interleukin-12 Expressing Dendritic Cells for the Treatment of Animal Model of Leukemia

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Residual cancer cells appearing in blood circulation reduce the effects of radiotherapy or chemotherapy in cancer patients. It has been well documented that cultured dendritic cells can be used as a powerful tool to induce immune response. In this study, we administered different manipulations of dendritic cells (DCs), including DCs pulsed with tumor cell lysate (TCL), transfected with adenoviral IL-12 vector (AdIL-12) and transfected with AdIL-12 after being pulsed with TCL, to determine whether improved DCs based immunotherapy can specifically suppress the metastasis of tumor cells. The results demonstrated that administration of engineered DCs that transfected with AdIL-12 after being pulsed with TCL to mice with leukemia had a better protective effect than that of DCs either pulsed with TCL or transfected with AdIL-12. Moreover, depletion of CD8⁺ cells in the engineered DCs administered leukemia mice reduced the protective effect. These results suggest that DCs modified with TCL and AdIL-12 can prolong survival time by enhancing the activity of cytotoxic T cells. Although more studies on the mechanisms are needed, cytokine genes engineered DCs provide a promising therapeutic potential on the murine model of leukemia. *Exp Biol Med* 234:952–960, 2009

Key words: dendritic cells; RL δ 1; leukemia; adenoviral vector; interleukin-12

Introduction

Leukemia is a cancer of the white blood cells that is disseminated by blood circulation. Because its features differ from solid tumors in targeting therapy, leukemia cells

might disseminate in blood circulation, which makes it more difficult to be a specific target than other tissues and organs. This reduces the therapeutic effects of radiotherapy or chemotherapy, which are the current methods of cancer treatment for cancer patients when residual cancer cells appear in blood circulation. Specifically targeting the cancer cells in the blood is important for lowering the risks of metastasis. There are natural mechanisms in tumor-immune surveillance, which defend against the spread of tumor cells. Recently, immune-editing hypothesis predicts that tumor cells that escape from elimination by the immune system generate the ability to reduce immunogenicity or an increased capacity to inhibit anti-tumor immune responses (1).

Dendritic cells (DCs) are potent antigen-presenting cells (APC) that can elicit a primary immune response and boost secondary immune responses to foreign antigens (2, 3). In a variety of settings, these specialized cells can induce both the generation and proliferation of specific CTL and T-helper cells via antigen presentation by MHC class I and class II molecules, respectively. Because of these properties, much attention has been directed towards the use of DCs in vaccine strategies for cancer.

DCs pulsed with tumor-associated antigens in various forms, including whole cell lysates (4, 5), peptides (6), proteins (7), RNA (8, 9) or DNA (10), have been studied for anti-tumor effects in experimental tumor models. Indeed, DC vaccination for tumors in experimental murine models has produced encouraging results (4–10). Furthermore, DCs from cancer patients lose effector functions, such as expressing low levels of HLA-DR and CD86 and inducing decreased interleukin-12 (IL-12) secretion *in vitro* (15).

To enhance such functions, genetic modified DCs have been applied and have demonstrated positive results in tumor inhibition in murine models (11–14). IL-12 is a heterodimeric cytokine produced by macrophages, mitogen stimulated- or EBV infected-B lymphocytes, keratinocytes, and probably dendritic cells, with important immunoregulatory functions both *in vitro* and *in vivo* (15). It directly stimulates activated NK and T cells to produce high

This study was supported by a grant from National Science Council.

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Received May 16, 2008.
Accepted April 14, 2009.

DOI: 10.3181/0805-RM-165
1535-3702/09/2348-0952\$15.00
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levels of IFN- γ , enhances their cytolytic activity, and promotes maturation of Th1 cells as well as IL-2-activated B cells (15, 16). In this study, we engineered DCs with tumor cell lysates (TCL) and AdIL-12 to enable DCs to present tumor antigens and secrete IL-12 at the same time, and determine if these can specifically suppress the metastasis of tumor cells.

Applying these engineered DCs in murine models of leukemia, RL δ 1 leukemia T cells injected into mice via their tail veins showed that survival time can be prolonged and tumor growth can be suppressed compared to that of a control group. When the CD8 $^{+}$ cells in tumor mice were depleted, the engineered DCs could not protect the mice from invading leukemia cells. In addition, flow cytometry analysis suggested engineered DCs have no significant differences on surface markers like CD80, CD86, MHC class II and CD11c compared to DCs alone.

In summary, engineered DCs can be powerful tools in suppressing tumor cell dissemination and metastasis in the circulation. CD8 $^{+}$ T cells play an important role in the suppression mechanism. Engineered DCs should be considered as a supporting tool to remove residual tumor cells in blood circulation after radiation therapy or chemotherapy.

Materials and Methods

Tumor Cell Lines and Animals. RL δ 1 is a cell line derived from radiation-induced T-cell leukemia in a Balb/c mouse and was kindly provided by Dr. Lih-Hwa Hwang (College of Medicine, National Taiwan University). The cells were maintained in Balb/c mice by *in vivo* passage. Briefly, RL δ 1 (1×10^6 cells/mouse) were injected intraperitoneally (i.p.) into Balb/c mice. Cells were harvested 10–14 days after inoculation and stored in liquid nitrogen until use. Upon infection, cells were thawed and grown in RPMI-1640 medium supplemented with 10% fetal calf serum (FCS; Gibco Laboratories, Grand Island, NY). Typically, animals injected s.c. with 1×10^5 of RL δ 1 died 40–50 days after inoculation, due to outgrowth of the tumor. Male Balb/c mice aged 6–8 and 10–12 weeks were used in these experiments. They were purchased from the animal center of the National Taiwan University Hospital (Taipei, Taiwan).

Preparation of Tumor Cell Lysate. RL δ 1 cells were re-suspended at 3.5×10^8 cells/ml in Hank's balance salt solution and disrupted by four freeze/thaw cycles (alternating dry ice/37°C water bath). The tumor cell lysates were centrifuged at $600 \times g$ for 10 min and the supernatant was collected and centrifuged again at $13,000 \times g$ for 60 min. The final supernatant was collected, filtered through a 0.2 μ m filter, and aliquoted into freezing vials to be stored at -70°C until needed. The supernatant was used as sources of soluble TCL.

Generation of Dendritic Cells from Bone Marrow Cultures. DCs were prepared as described previously (29–31). Briefly, erythrocyte-depleted mouse bone marrow

cells were cultured in RPMI-1640 medium supplemented with 5% FCS with murine rGM-CSF (500 U/ml) and rIL-4 (1000 U/ml) (Pepro Tech Inc., Rocky Hill, NJ) for 8 days. The purity of the DCs ($>70\%$) were collected and analyzed by flow cytometry examining the expression of MHC class II, CD80, CD86, CD11c, DEC-205, and 33D1.

Cytokine-Expressing Adenovirus Vectors. Cytokine- and green fluorescence protein (GFP)-expressing adenovirus vectors were obtained. Construction of the single-chain IL-12 gene was performed as previously described (17, 18). The p40 and p35 subunits of the murine IL-12 gene were connected by a linker that generated the single-chain IL-12 gene. Mouse IL-12 (AdIL-12) gene was inserted into the pAdhPGK vector. The pAdhPGK vector carried no cytokine transgene as a control (Ad-mock). The viral stocks were prepared by 293 cells transfected with IL-12 expressing or mock vector. We collected the supernatant of vector transfected 293 cells after amplification. The cells were lysed by repeated thawing and freezing. After CsCl purification and dialysis, the viral titer was titrated by standard protocol. The final viral titer was 10^{12} pfu/ml. We infected 5×10^5 DCs with different titer of virus and got the optimized titer of virus infection— 1.5×10^7 pfu. So the MOI equals to 30. The transduction efficiency of adenovirus was from 63% to 80% (mean $\pm$ SD is $70\% \pm 6.46$) analyzed by flow cytometry in 5 different independent experiments.

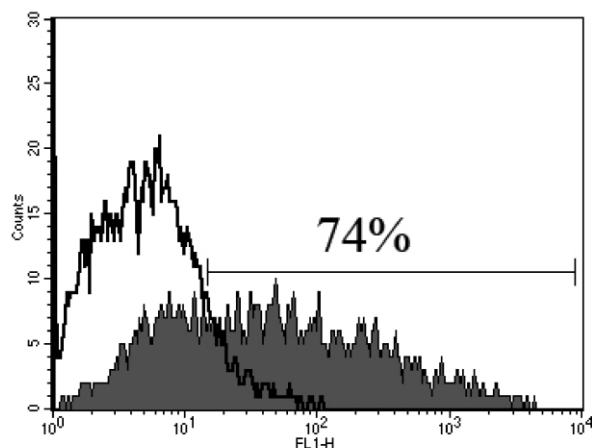
Preparation of Engineered Dendritic Cells. DCs were cultured as described above. At day 4, DCs were seeded into 24-well culture plate with 5×10^5 cell number. TCL was added at 50 μ g/well into the culture plate. The next day, AdIL-12 with MOI of 30 were added into the wells with TCL-pulsed DCs. DCs that were transfected with adenoviral vectors after feeding with TCL were defined as engineered DCs. On day 6, these engineered DCs were collected for experiments.

ELISA for the Determination of Mice IL-12 Cytokines. To measure the amounts of cytokine secreted from the engineered DCs, commercially available enzyme-linked immunosorbent assay (ELISA) kits for mIL-12 p70 (R&D, UK) were used. Procedures followed were described in the manufacturer's instructions. The concentration of cytokines in the sample was obtained by interpolation from the standard curve.

In Vivo Immunization and Tumor Challenge. Balb/c mice were immunized intravenously (i.v.) in the tail vein with engineered DCs twice at seven-day intervals. To study the preventive effect, the mice were re-challenged one week after the last immunization with a lethal dose of 1×10^4 RL δ 1 viable tumor cells by i.v. injection into the tail vein. Alternatively, the mice immunization with 5×10^3 RL δ 1 viable tumor cells by i.v. injection into the tail vein, and then treated with engineered DCs at the same day to determine the therapeutic effects. During the follow-up period, the mice were monitored for the survival time.

Cell-Mediated Cytotoxicity Assays. Spleens iso-

(A)



(B)

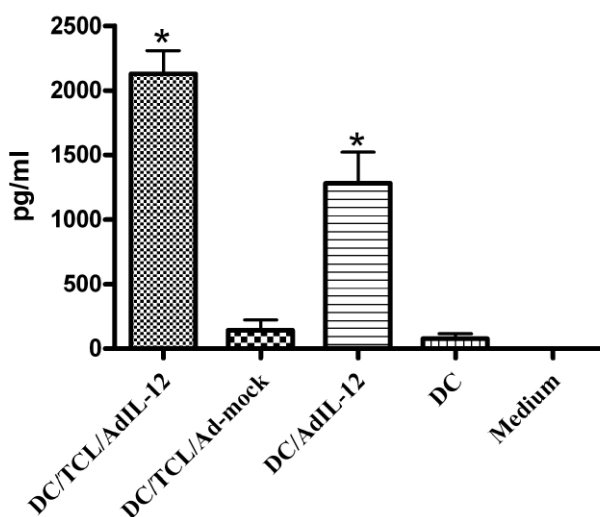


Figure 1. Transfection efficiency of DCs infected with adenoviral GFP (AdGFP) or IL-12 (AdIL-12) vector. (A) The DCs were transfected with AdGFP that express a fluorescent protein, at day 6 and analyzed by flow cytometry as described in the Materials and Methods section. DCs that had been transfected with AdGFP (full grey) were compared to DCs alone (solid curve). (B) Levels of IL-12 expression in engineered DCs (DC/TCL/AdIL-12), TCL pulsed DCs transfected with mock (DC/TCL/Ad-mock), DCs transfected with AdIL-12 (DC/AdIL-12), and DCs alone. Results are the mean $\pm$ SD of duplicated wells. Asterisk is a significant difference when compared to DCs alone (* $P < 0.05$). The data shows one of five independent experiments with similar results.

lated from the Balb/c mice received different treatments seven days after challenging with tumors. *In vitro* stimulation was performed by incubating 5×10^6 splenocytes with 1×10^5 irradiated RL δ 1 cells per well in 24-well plates for 5 days at 37°C in the presence of 10 U/ml recombinant human IL-2. Target cells were allowed to be incubated with ^{51}Cr for 1 hour, and then washed extensively with RPMI medium. The labeled target cells (1×10^4) were mixed with effector cells at effector/target (E/T) ratios of 100:1, 50:1, 10:1, and 5:1 in 96-well U-bottomed plates.

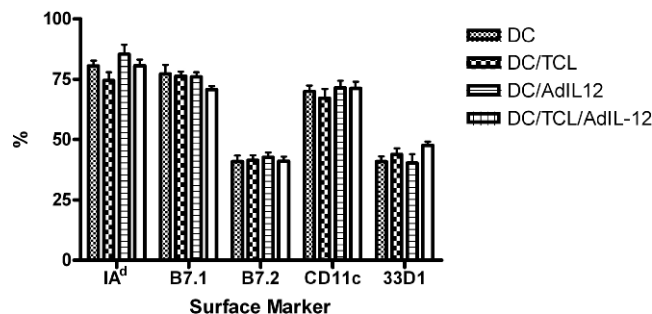


Figure 2. Surface marker expression percentage of engineered DCs. The engineered DCs that DCs pulsed with TCL and transfected with AdIL-12 or Ad-mock were immunostained with fluorescently conjugated antibodies including anti-MHC class II (IA^d), CD80 (B7.1), CD86 (B7.2), CD11c and 33D1 and analyzed by flow cytometry as described in the Materials and Methods section. The engineered DCs stained with different fluorescence conjugated antibodies, and compared to isotype control. The surface marker expression levels were shown as a percentage of gated. The average of surface marker expression levels from 3 independent experiments summarized as mean $\pm$ SD.

The mixtures were incubated at 37°C for 4 hours, and the released ^{51}Cr radioactivity was measured in 100-ml aliquots of the supernatants. The percentage of specific lysis was calculated using the following formula:

$$\% \text{ specific lysis} = \frac{\text{cpm}_{\text{exp}} - \text{cpm}_{\text{bkgd}}}{\text{cpm}_{\text{max}} - \text{cpm}_{\text{bkgd}}} \times 100.$$

Each assay was performed in triplicate, and spontaneous release was $<20\%$ of the maximal release by detergent in all assays.

Winn Test. Spleen cells were isolated from mice that were treated with DCs transfected with AdIL-12 or Ad-mock after being pulsed with TCL or DCs alone. One week after the second immunization of engineered DCs from untreated mice, naïve mice were injected s.c. with mixtures of 1×10^7 splenocytes described above and 10^6 parental RL δ 1 cells. During the follow-up period, the mice were tracked for tumor size and survival time. Primary tumor areas were measured twice a week using mechanical calipers. Tumor volumes were calculated by the formula $v = \pi ab^2/6$, where a is the longest diameter and b is the next longest diameter perpendicular to a (19, 20).

Statistical Analysis. All of the results were expressed as the mean $\pm$ standard error of the mean (SEM) and evaluated with a one-way analysis of variance (ANOVA), followed by an unpaired two-tailed Student's t test for comparison between the two groups.

Results

Transfection Efficiency of Dendritic Cells with Adenoviral IL-12. DCs transfected with adenoviral vectors after being pulsed with TCL were analyzed by flow cytometry and determined by ELISA. To determine the efficiency of the adenoviral vector transfection, we used adenoviral-GFP vectors that expressed fluorescent proteins.

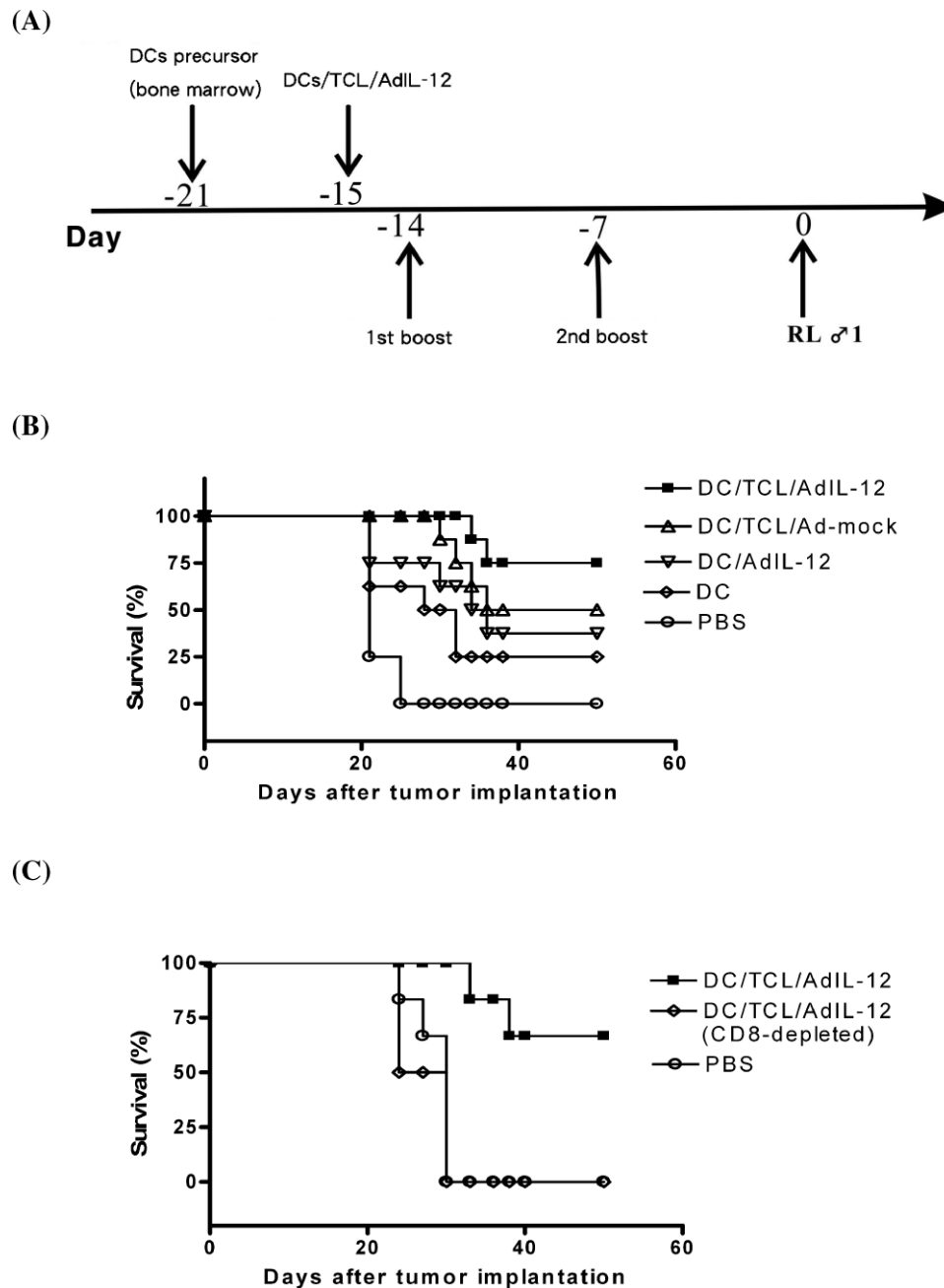
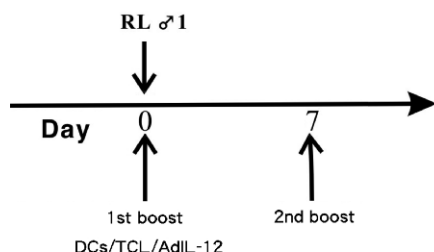


Figure 3. Preventive effects of leukemia model with engineered DCs. (A) The scheme for administering engineered DCs or DCs alone to mice before tumor implantation. (B) Preventive effects on mice vaccinated with engineered DCs before leukemia cells i.v. injection. Each group consisted of 8 mice. (C) Anti-tumor effects on mice vaccinated with engineered DCs were depleted CD8⁺ T cells before tumor transplantation. Each group consisted of 8 mice.

The control cells were normalized between 10^1 and 10^2 in fluorescence intensity. The transduction efficiency of adenovirus is from 63% to 80% (mean $\pm$ SD is $70\% \pm 6.46$) analyzed by flow cytometry in 5 different independent experiments. As Figure 1A shows, 74% of the cells expressed fluorescent proteins compared to DCs alone. Different doses of adenoviral vectors were tried during the DC transfections, and we finalized an “optimal dose” of 30 MOI (data not shown). Every transfection was monitored for cell viability by adding trypan blue and scoring live vs.

dead cells in a haemocytometer. The viability of DCs transfected with adenoviral vector was as much as 99% with no difference among different groups. The cell culture supernatants of engineered DCs were collected to determine IL-12 expression level by ELISA. Both TCL pulsed-DCs and DCs alone infected with AdIL-12 expressed significantly higher level of IL-12 cytokines than those with DCs (Fig. 1B). Interestingly, IL-12 expression levels were significantly higher when pulsed with TCL than DCs infected with AdIL-12 (Fig. 1B). This suggested that the

(A)



(B)

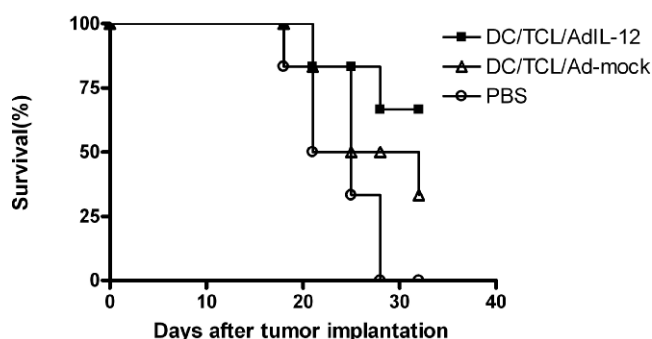


Figure 4. Treatments of leukemia model with engineered DCs. (A) The scheme for administering TCL pulsed-DCs transfected with AdIL-12 of mock to mice after tumor implantation. (B) The therapeutic effects on mice treated with TCL pulsed-DCs transfected with AdIL-12 (■, DC/TCL/AdIL-12) or mock (△, DC/TCL/Ad-mock), or PBS (○, PBS) after leukemia cells i.v. injection. Each group consisted of 6 mice.

engineered DCs made by AdIL-12 transfection and pulsed with TCL had good transfection efficiency and higher IL-12 expression levels.

Evaluation of Engineered Dendritic Cells Surface Marker Expression. Engineered DCs were routinely immunostained with fluorescent conjugated antibodies and analyzed for surface marker expression by flow cytometry. To compare the condition of engineered DCs with different manipulations before injection into the mice, surface markers—including MHCII, CD80, CD86 and CD11c—were analyzed. Isotype antibodies were used as controls. Figure 2 showed the mean percentage of 3 independent experiments for four different manipulations of DCs, including DCs infected with AdIL-12 after being pulsed with TCL, DCs infected with AdIL-12 only, DCs pulsed with TCL only and DCs that expressed high levels of MHCII and CD80 and CD86 molecules. Thus, the protocols of engineered DCs used in this study did not significantly alter the normal repertoire of surface antigens, particularly those involved in antigen presentation and co-stimulatory signaling pathways.

Systemic Anti-Tumor Immunity Induced by

Engineered DCs. To determine whether the protective immunity elicited by engineered DCs injection also exerted any therapeutic effects, and whether the effects were long-lasting and superior to DCs pulsed with TCL, we evaluated the four different manipulations of DCs. Mice were immunized with engineered DCs twice by i.v. injection at seven day intervals. On day 7 after the second immunization, the mice with engineered DCs received lethal dose of RL♂1 tumor cells and the survival rate was followed to determine the systemic anti-tumor immunity (Fig. 3A). As Figure 3B shows, mice immunized with engineered DCs, DCs pulsed with TCL, and DCs infected with AdIL-12 had significant effects in prolonging survival time compared to mice treated with PBS or DCs alone. Compared to mice treated with DCs pulsed with TCL or DCs infected with AdIL-12, IL-12 engineered DCs pulsed with TCL did prolong survival rate better than other groups (Fig. 3B). The results showed that the anti-tumor effects of DCs based on a tumor vaccine were improved by AdIL-12 transfection and pulsed with TCL. Furthermore, mice treated with engineered DCs lost the anti-tumor effects when the CD8⁺ cells were depleted before the tumor was transplanted (Fig. 3C). To determine the therapeutic effects of engineered DCs, mice were immunized with the manipulated DCs at the same day as mice were injected i.v. with tumor cells, and follow-up monitored the survival time (Fig. 4A). The anti-tumor effects of tumor mice treated with AdIL-12 transfected TCL pulsed-DCs showed better survival time than control groups (Fig. 4B). However, the delivery of AdIL-12 transfected TCL pulsed DCs 5 or 7 days after tumor cells injection did not show any beneficial effect for both suppression of tumor growth or survival time.

Cellular Immunity Responsible for the Mice Treated with Engineered DCs.

Next, we investigated whether the protective immunity was cell-mediated using the Winn test. The results revealed that the splenocytes derived from different manipulation of DCs-treated mice conferred various degrees of protection on the mice (Fig. 5A). The splenocytes from mice treated with engineered DCs had better protection and longer survival time than splenocytes from mice treated with TCL pulsed-DCs transfected with mock, DCs alone, or PBS (Fig. 5B). Figure 6 illustrated the results of CTL assays performed 1 week after the second immunization. Control animals treated with DCs alone or untreated exhibited low levels of CTL activity (Fig. 6A). However, the specific lysis of DC/TCL/AdIL-12 (64.9%) is significantly higher than that of DC/TCL/Ad-mock (53.4%) (Fig. 6A). Significantly higher levels of tumor-specific cytotoxicity were observed in the groups of mice treated with engineered DCs compared to mice treated with TCL pulsed-DCs transfected with mock vector. Most of the CTL activities were mediated by CD8⁺ T cells as indicated by inhibition of lysis after treatment of spleen cells with anti-CD8 antibodies and complement (Fig. 6B). Furthermore, the CTL activities observed were tumor specific because the cytotoxic T cells displayed no activity

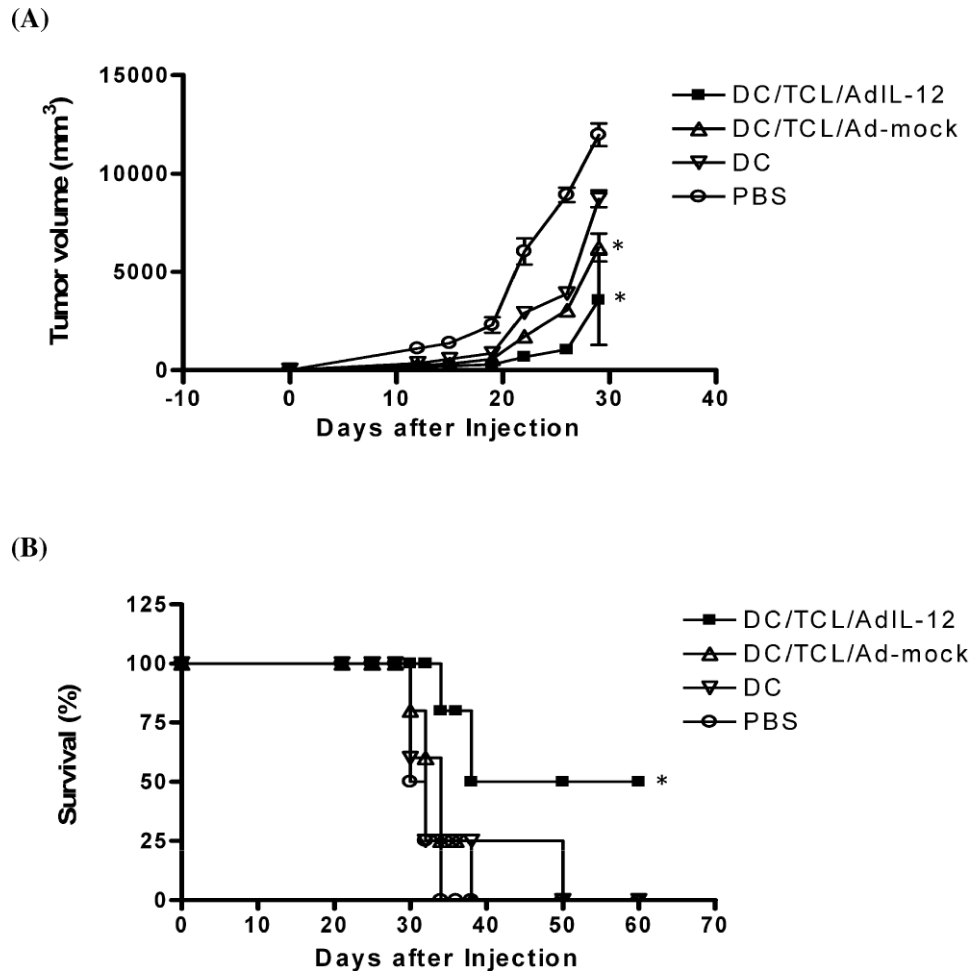


Figure 5. Cellular immunity responsible for antitumor activity. Winn test. The splenocytes (1×10^7) were isolated from mice treated with TCL pulsed-DCs transfected with AdIL-12 (■, DC/TCL/AdIL-12) or mock (△, DC/TCL/Ad-mock), DCs alone (▽, DC) or PBS (○, PBS) 1 week after second vaccination and mixed with 1×10^6 parental RL δ 1 cells. The mixtures were injected s.c. into naïve Balb/c mice. (A) Tumor size measured starting on day 12 after mixture injection. Tumor growth was inhibited significantly with splenocytes isolated from mice treated with TCL pulsed-DCs transfected with AdIL-12 or Ad-mock, and DCs therapy alone in comparison to that from untreated (* $P < 0.05$). (B) The survival rates of different groups were monitored. Each group was composed of 5 mice (* $P < 0.05$).

toward a Balb/c-derived mammary tumor cell line JC cells (Fig. 6C). Although the data suggested CD8⁺ T cells were critical of killing tumor and suppressing tumor growth both *in vitro* and *in vivo*. The mice treated with DC/TCL/Ad-mock also showing good cytotoxic activity (53.4%) might be due to the potency of antigen presentation and IL-12 production of DCs. In addition, we also assayed both the level of natural killer (NK) cells and IFN- γ in the mice; however, no significance was noted among the groups (data not shown).

Discussion

Tumor metastasis is a complex process that involves many factors (21–24). Although a variety of mechanisms are involved, blood circulation is still regarded as the major route of tumor cells dissemination. It is difficult to specifically target residual tumor cells in the blood circulation, especially in leukemia, and immunotherapy is considered to be a safe and efficient treatment so far. To

develop an effective immunotherapy for the prevention of metastasis and recurrence of tumor cells becomes a major task in designing a novel treatment for cancers.

IL-12 has been suggested to be critical of bridging the innate and adaptive immunity. In addition to its effects in the priming of Th1 cell responses and stimulating IFN- γ production by T and NK cells, it is also important for CD8⁺ cells differentiation (15, 25–27). Some studies have already demonstrated that IL-12 has the effect of anti-angiogenesis and further suppression of metastasis (28). This study demonstrated that AdIL-12 modified DCs pulsed with TCL induces significant anti-tumor immunity that is basically T-cell-mediated in a murine T-cell leukemia model. The anti-tumor immunity presented long-lasting protective effect from leukemia tumor cell dissemination in the blood circulation. TCL pulsed-DCs transfected with AdIL-12 secrete higher levels of IL-12 than AdIL-12 modified DCs without TCL (Fig. 1B). Although the mechanisms are not yet to be defined, it is possible that high levels of IL-12

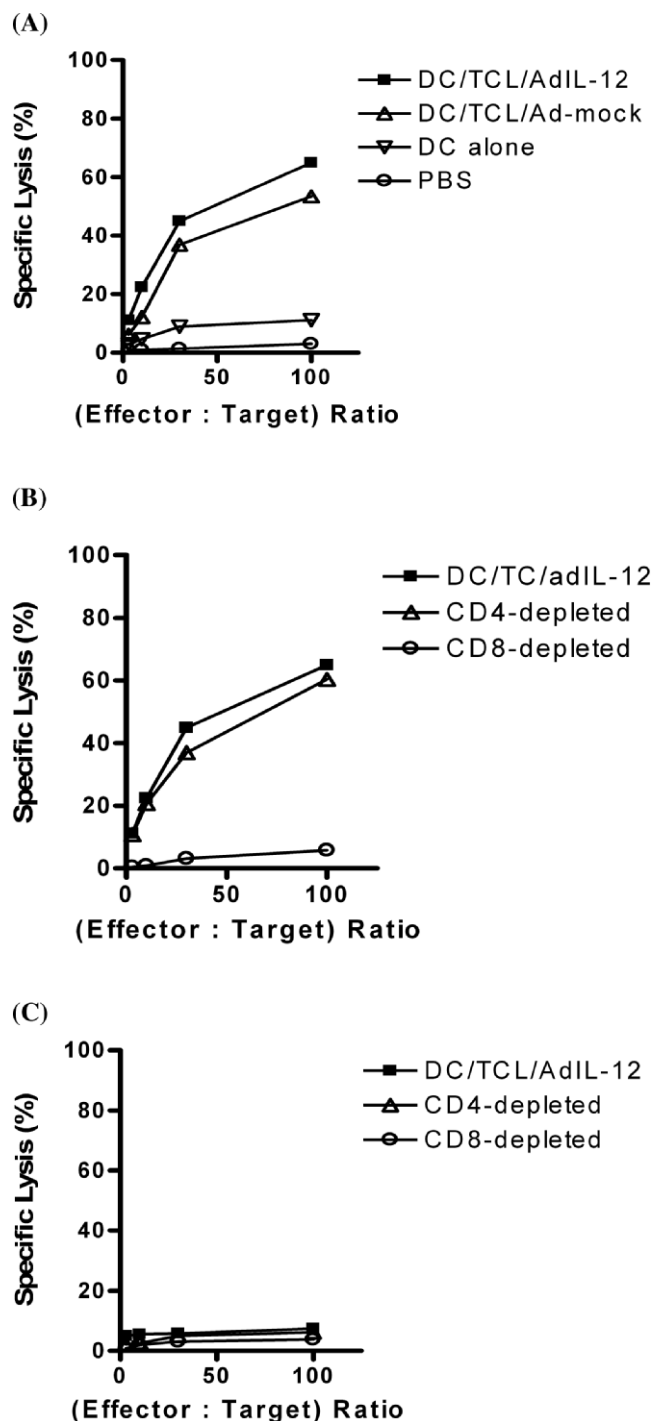


Figure 6. Induction of tumor-specific cytotoxic T cells in animals vaccinated with engineered DCs. (A) The splenocytes were isolated from mice that were treated with TCL pulsed-DCs transfected with AdIL-12 (■, DC/TCL/AdIL-12) or mock (▲, DC/TCL/Ad-mock), DCs alone (▼, DC) or PBS (○, PBS) 1 week after the 2nd vaccination. (B) The splenocytes from the group of mice treated with TCL pulsed-DCs transfected with AdIL-12 were depleted CD4⁺ (▲) or CD8⁺ (○) T cells with antibodies before CTL activity assay. They were stimulated *in vitro* with irradiated RL δ 1 cells for 5 days and, then, the cytolytic activity was assayed at the indicated E/T ratios. (C) As a negative control, a BALB/c-derived mammary tumor cell line, JC, was used as a CTL target using the splenocytes from mice treated with DC/TC/AdIL-12. To determine the cell types responsible for CTL activity, antibodies against CD4 (GK1.5) or CD8 (3.155) plus complement were performed to deplete CD4⁺ or CD8⁺ cells from splenocytes.

production by cancer vaccines can induce optimal anti-tumor immunity.

Most recently, DCs have been found to play a critical role in the induction and modulation of immune responses for treatment of leukemia (11, 29–31). In addition, studies also suggested that myeloid and lymphoid dendritic cells exert completely different effects on T helper cell development. DCs have been suggested to be “professional” antigen presenting cells, which can process and present antigens *in vivo* (36). It has also been demonstrated that DCs can be grown *in vitro* with the addition of cytokines such as GM-CSF, TNF- α and IL-4 (32). Most studies have demonstrated that *in vitro* cultured DCs are able to induce both MHC class I and MHC class II-restricted immune responses in several models. Thus, DCs have been used for inducing effective immune responses against tumors (3–10). However, the major problem encountered in the naked DNA immunization is the immunogenicity of the antigen, which DCs may overcome to elicit the better required immune response (33).

Inserting a foreign gene into an adenovirus vector has been reported to express high levels of foreign antigen expression. One study showed that human DCs infected with adenovirus can suppress proliferation of autologous and allogeneic T cell, and DCs infected with adenovirus enhance immunostimulatory properties (34). We modified the DCs for inducing cytokine protein expression using an adenoviral vector inserted with the IL-12 gene. The high percentage of positive expression proves that it is good for modulating protein expression by DCs. The same phenomena could be noted in the expression of IL-12 from DC culture supernatant after AdIL-12 infection *in vitro* assay (Fig. 1B). Using Ad-GFP infected mouse bone marrow derived DCs, our reported infection rate reached 70% at an MOI of 10 (35).

DCs produced IL-12 is known to polarize T-lymphocytes toward Th1 development, which is considered to be the most effective in anti-tumor immune response (36). In addition, IL-12 has been found to inhibit angiogenesis of tumors, which is the most important element in tumor metastasis (37). Mice that were treated with engineered DCs secreted with high levels of IL-12 not only had prolonged survival times but were protected from leukemia cells forming solid nodules on the liver and lungs, which were the major sites of our tumor model localized by i.v. injection. Furthermore, we also demonstrated that AdIL-12 improved the anti-tumor effects of DCs based tumor vaccines, not only preventive but also therapeutic (Fig. 3B, 4B). However, the delivery of AdIL-12 transfected TCL pulsed DCs 5 or 7 days after tumor cells injection did not show any beneficial effect for both suppression of tumor growth or survival time (data not shown).

Despite the detection of tumor-specific CTL activities and the CD8⁺ T cells in the mice treated with engineered DCs, the immune cells did not confer protective effects upon the animals transplanted with lethal doses of tumor

cells prior to being vaccinated with engineered DCs. However, lower tumor cells transplanted mice treated with engineered DCs simultaneously prolonged the survival time compared to control group (Fig. 4B). The question then arises as to why tumors were not cleared in this group of mice, and how the tumors manage to grow in the vicinity of activity of activated CD8⁺ T cells. In addition to CD8⁺ cells, the innate immune response such as IFN- γ production and NK cells could also be recruited in the process of anti-tumor immune response by IL-12. We have assayed the spleen NK cells population in DCs treated mice with anti-DX5/CD3 antibody by flow cytometry and serum IFN- γ levels, respectively. The results suggested that there is no significant difference not only in spleen NK population but also serum IFN- γ between mice treated with engineered DCs or DCs alone (data not shown). However, the results did show better survival time of mice treated with IL-12 secreting DCs pulsed with TCL. There might be other mechanisms such as anti-angiogenesis involved in the therapeutic effect of IL-12 and more studies are needed to clarify the effects. The data suggested that the treatment of IL-12 and TCLs modified DCs could specifically induce effective anti-tumor immune response.

Several possible mechanisms might be suggested to escape from immune attacks; one is the inhibitory cytokines, TGF- β and IL-10, secreted by our tumor model. Before the anti-tumor immune response was induced in mice treated with engineered DCs, the lethal dose of tumor cells proliferated too large to be suppressed by the immune response. However, the anti-tumor effects induced by the engineered DCs before tumor challenged was enough to ward against tumor growth.

In conclusion, this study demonstrates that IL-12 secreting DCs pulsed with TCL can induce specific immune responses against tumor cells in blood circulation that mimic leukemia cell dissemination. However, this kind of approach is still not effective for the long established tumors, and it might be due to the efficiency of tumor killing by cytotoxic T cells. Our observations imply that strategies to reverse the possible immune-suppressive conditions in tumor-bearing mice can be as important as the strategies used solely to augment immune responses. Hence, immunotherapy with cytokine engineered dendritic cells can function as an adjuvant treatment, providing systemic and long-lasting anti-tumor effects that can prevent relapses. More studies are needed to develop effective treatment regimens for malignant tumors along these lines.

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