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RESEARCH ARTICLE

Induction of antitumor immunity against mouse carcinoma by baculovirus-infected dendritic cells

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A dendritic cell (DC) vaccine strategy has been developed as a new cancer immunotherapy, but the goal of complete tumor eradication has not yet been achieved. We have previously shown that baculoviruses potently infect DCs and induce antitumor immunity against hepatomas in a mouse model. Baculovirus-infected, bone marrow-derived DCs (BMDCs) display increased surface expression of costimulatory molecules, such as CD80, CD86 and major histocompatibility complex (MHC) classes I and II, and secrete interferons and other proinflammatory cytokines. In this study, we evaluated the induction of antitumor immunity in mice by baculovirus-infected BMDCs against lung cancer and melanoma. After treatment with baculovirus-infected BMDCs, murine lung tumors caused by Lewis lung carcinoma (LLC) cells were significantly reduced in size, and the survival of the mice was improved. In addition, experiments using a melanoma mouse model showed that baculovirus-infected BMDCs inhibited tumor growth and improved survival compared with controls. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and creatinine levels remained normal in baculovirus-infected BMDC-treated mice. Our findings show that baculovirus-infected DCs induce antitumor immunity and pave the way for the use of this technique as an effective tool for DC immunotherapy against malignancies.
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Keywords: dendritic cells; natural killer cells; T cells; tumor Immunity

INTRODUCTION

Dendritic cells (DCs) play a major role as professional antigen-presenting cells in the activation of natural killer (NK) cells, T cells and B cells. In particular, DCs are important in the initiation of innate and adaptive immune responses against tumors and invading pathogens. Recently, DC vaccination has been reported as a new immunotherapeutic strategy for the treatment of cancers.[1] However, very few clinical studies using DC immunotherapy have been reported, and those studies demonstrated insufficient efficacy.

The baculovirus *Autographa californica* multiple nuclear polyhedrosis virus, is an enveloped insect virus that has a 130-kb double-stranded circular DNA genome.[2] Baculoviruses have long been used as biopesticides[3,4] and recombinant protein expression systems.[5,6] Recent research has focused on the use of baculoviruses as vectors in gene therapy because of (1) their ability to infect, but not replicate in, mammalian cells; (2) their low cytotoxicity; and (3) their ability to carry large foreign genes into their genome.[7–10] In several reports, baculoviruses were developed as vaccines against pathogens.[11–15] Abe *et al.* reported that baculovirus-infected host cells produced type I interferons and proinflammatory cytokines through Toll-like receptor 9 and interferon regulatory factor 7 signaling.[16,17] Schütz *et al.* reported that baculovirus-infected, human monocyte-derived DCs expressed cell-surface activation markers and produced tumor-necrosis factor alpha (TNF-α).[18] In addition, Hervas-Stubbs *et al.* reported that baculoviruses strongly induced the secretion of type I interferon and proinflammatory cytokines in mice.[19] Conventional splenic DCs and plasmacytoid DCs displayed upregulation of surface major histocompatibility complexes (MHCs) and costimulatory molecules in response to baculovirus infection *in vivo*. Jordan *et al.* demonstrated that vaccination with insect cells infected with recombinant, baculovirus-encoding peptide–MHC complexes generated peptide-specific cytotoxic T-cell responses and antitumor immunity.[20] We have previously described the induction of antitumor effects using wild-type baculovirus in a mouse model of hepatoma.[21] In particular, DCs were more potently infected by baculovirus than were other immune cells, and the DCs underwent activation and maturation. Baculovirus-infected bone marrow-derived dendritic cells (BMDCs) (BV-DCs) showed upregulation of costimulatory molecules, such as CD80, CD86 and MHC classes I and II, and increased secretion of interferons and other proinflammatory cytokines.[22]

In this study, we showed that the inoculation of BV-DCs induced efficient $CD8^+$ T cell- and NK cell-dependent, $CD4^+$ T cell-independent antitumor immunity. Furthermore, BV-DC administration did not cause any functional damage to the mouse liver or kidneys. Our results demonstrate that BV-DC-induced antitumor immunity *in vivo* has strong potential as a future DC immunotherapy against various malignancies.

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MATERIALS AND METHODS

Mice, cell lines and reagents

Female C57BL/6 mice (6 weeks old) were purchased from Nippon SLC (Hamamatsu, Japan) and maintained under humane conditions according to the rules and regulations of our institutional committee. *Spodoptera frugiperda* (Sf-9) cells were cultured at 27 °C in Sf-900 II medium (Invitrogen, Carlsbad, CA, USA). Lewis lung carcinoma (LLC), B16F10 and EL4 cells were obtained from the RIKEN Cell Bank (Wako, Saitama, Japan). LLC and EL4 were maintained in Dulbecco's modification of Eagle's medium (Invitrogen) supplemented with 10% fetal calf serum (Sigma Chemical, St Louis, MO, USA), 100 U/ml penicillin, and 100 μg/ml streptomycin (Sigma Chemical). B16F10 cells were maintained in RPMI-1640 (Invitrogen) supplemented with 10% fetal calf serum. Synthesized phosphorothioate-stabilized mouse type A CpG oligodeoxynucleotides (CpG-A: ODN-D19) (GGTGCATCGATGCAGGGGGG) were purchased from Hokkaido System Science (Sapporo, Hokkaido, Japan). Recombinant murine granulocyte–macrophage colony-stimulating factor, murine IL-4 and human IL-2 were obtained from PeproTech EC Ltd (London, UK).

Purification of wild-type baculovirus

Wild-type baculovirus was purchased from BD Biosciences (San Jose, CA, USA) and propagated in Sf-9 cells in Sf-900 II medium. The baculovirus was purified as previously described,[21] and the virus titer was determined by the plaque assay.

Generation of murine BMDCs

Murine BMDCs were generated as described previously.[23] Briefly, bone marrow cells were harvested from the tibiae and femurs of C57BL/6 mice and depleted of red blood cells using red blood cell lysis buffer (Sigma Chemical). Bone marrow cells were cultured in RPMI-1640 medium containing 10% fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine (Invitrogen) and 50 μM 2-mercaptoethanol (Invitrogen), supplemented with 20 ng/ml each of murine granulocyte–macrophage colony-stimulating factor and IL-4. On days 3 and 5, the culture medium was replaced with fresh medium that was supplemented with murine granulocyte–macrophage colony-stimulating factor and IL-4 at the same concentration. On day 7, non-adherent and loosely adherent cells were collected and positively selected with anti-mouse CD11c microbeads (Miltenyi Biotech, Bergisch Gladbach, Germany).

Baculovirus infection of BMDCs

BMDCs (1×10^6 cells) were infected with wild-type baculovirus at a multiplicity of infection (MOI) of 50 or incubated with CpG (1 μM) as a control for 1 h at 37 °C. Next, the cells were washed twice with sterile physiological saline and cultured for 5 h at 37 °C. The cells were then harvested, thoroughly washed twice with physiological saline and resuspended in 100 μl of physiological saline. To determine the presence of viral protein in BMDCs, cells were collected at 1 and 3 h after infection and lysates were assayed by western blot analysis using anti-gp64 monoclonal antibody that targeted the baculoviral envelope. The result indicated the complete degradation of baculovirus 3 h post-infection in BMDCs (Supplementary Figure 1).

DC-induced immunity against LLC *ex vivo*

LLC cells (1×10^6 cells/mouse) were intravenously injected into mice on day 0, followed by intravenous injection of (1×10^6 cells/mouse) of immature BMDCs (iDCs), CpG (1 μM)-treated BMDCs (CpG-DCs), wild-type BV-DCs (MOI=50) or (5×10^7 plaque-forming unit (pfu)/mouse) of wild-type baculovirus on day 3. Twenty-eight days after injection, all mice were killed, and cancer nodules on both lungs were counted. Lung tissue was then fixed with formalin (10%) and embedded in paraffin. The paraffin sections were stained with hematoxylin and eosin.

Cytotoxicity assay against LLC

LLC cells (1×10^6 cells/mouse) were intravenously injected into mice on day 0, followed by intravenous injection (1×10^6 cells/mouse) of iDCs, CpG-DCs or BV-DCs (MOI=50) on day 3. Twenty-eight days after injection, the mice were killed, and splenocytes were harvested. Splenocytes were cocultured with target cells (LLC or EL4) after treatment with mitomycin C (10 μg/ml) at effector/target ratios of 100 : 1, 50 : 1 and 25 : 1 in the presence of recombinant human IL-2 (100 IU/ml). After 5 days, the cytotoxicity was assessed using the CytoTox 96 Non-Radioactive Cytotoxicity Assay Kit (Promega, Madison, WI, USA) according to the manufacturer's protocol.

Ex vivo DC therapy against B16F10 cells

B16F10 cells (1×10^6 cells/mouse) were subcutaneously injected into mice on day 0, followed by intratumoral injections (1×10^6 cells/mouse) of iDCs, CpG-DCs or BV-DCs (MOI=50) on days 10, 17 and 24. Mice were injected with each type of BMDCs when the tumor diameters reached 7–9 mm. The tumor volume was measured every 3 days.

Cytotoxicity assay against B16F10

B16F10 cells (1×10^6 cells/mouse) were subcutaneously injected into mice on day 0, followed by intratumoral injection (1×10^6 cells/mouse) of iDCs, CpG-DCs or BV-DCs (MOI=50) on day 10. Seventeen days after injection, the mice were killed, and splenocytes were harvested. Splenocytes were cocultured with target cells (B16F10 or EL4) after treatment with mitomycin C (10 μg/ml) at effector/target ratios of 100 : 1, 50 : 1 and 25 : 1 in the presence of recombinant human IL-2 (100 IU/ml). After 5 days, the cytotoxicity was assessed using the CytoTox 96 Non-Radioactive Cytotoxicity Assay Kit (Promega) according to the manufacturer's protocol.

In vivo depletion of $CD4^+$, $CD8^+$ or NK cells

To deplete $CD4^+$ and $CD8^+$ T cells *in vivo*, mice were intraperitoneally injected with the ascites fluid (100 μl/mouse) from $CD4^+$ or $CD8^+$ hybridoma-bearing mice (clone GK1.5 or 2.43) on days 6, 9, 12, 15, 18 and 21 after tumor inoculation. To deplete NK cells, anti-asialo GM1 antibody (50 μl/mouse; Wako Pure Chemical Industries, Osaka, Japan) was intraperitoneally injected on days 6 and 9 after tumor inoculation. Flow cytometry analysis confirmed >90% depletion of the target cells.

Measurement of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and creatinine

Wild-type baculovirus (5×10^7 pfu/mouse) or BV-DCs (1×10^6 cells/mouse) were intravenously injected into mice. After 24 h, the sera were collected, and AST, ALT and creatinine levels were measured using the Transaminase CII-Test Wako (Wako Pure Chemical Industries) and the Creatinine Parameter Assay kits (R&D systems, Minneapolis, MN, USA) according to the manufacturer's protocols.

Statistical analyses

Statistical analyses were performed using Student's *t*-test. The mouse survival rates were analyzed using the log-rank test.

RESULTS

Antitumor effects of BV-DCs against lung cancer

Baculovirus infection of mouse splenic or BMDCs upregulated the expression of activation markers and the secretion of interferon-α and proinflammatory cytokines.[19,21,22] We have previously described the induction of antitumor immunity by wild-type baculovirus in a mouse hepatoma model. We also showed that BV-DCs upregulated the expression of MHC and costimulatory molecules and increased the secretion of type I interferons and proinflammatory cytokines.[22] Moreover, NK cells or T cells cocultured with BV-DCs produced interferon-γ and demonstrated increased cytotoxicity and proliferation. Here, we first evaluated the effects of antitumor immunity against murine lung cancer. The experimental schedule is shown in Figure 1a. Mice were inoculated with LLC cells and immunized 3 days later with iDCs, CpG-DCs, BV-DCs or wild-type baculovirus. CpG oligodeoxynucleotides and baculovirus were recognized by Toll-like receptor 9, and CpG-stimulated DCs showed upregulation of MHC and costimulatory molecules and increased secretion of proinflammatory cytokines, except type I interferons.[16,22,24] Twenty-eight days after immunization, the mice were killed and evaluated for lung tumors. In comparison with non-immunized or iDC-immunized mice, tumor nodules in BV-DC-immunized mice were reduced to nearly the same extent as those in CpG-DCs and wild-type baculovirus-immunized mice (Figure 1b and c). As shown in Figure 1d, histological examination of the tumor tissues in the tumor-bearing, non-immunized or iDC-immunized mice showed bleeding and necrosis of the tumor tissues with partial infiltration of neutrophils and lymphocytes, but BV-DC- and CpG-DC-immunized mice displayed normal lung histology without tumor formation. Survival was also prolonged in BV-DC-immunized mice as compared with that in the other groups (Figure 2a). Cytotoxicity was assessed for each group of mice, and the splenocytes of BV-DC-immunized mice demonstrated upregulation of cytotoxicity against LLC cells as compared with the other groups (Figure 2b). NK cell-resistant EL4 cells were used as target cells in a control experiment, and negative results were obtained (Figure 2c). These results indicate that BV-DC-induced antitumor activity against mouse lung cancer was due to the cytotoxic effects of both NK and $CD8^+$ T cells.

Antitumor effects of BV-DCs against melanoma

We next examined whether the BV-DCs could induce antitumor immunity against established subcutaneous tumors in mice. The

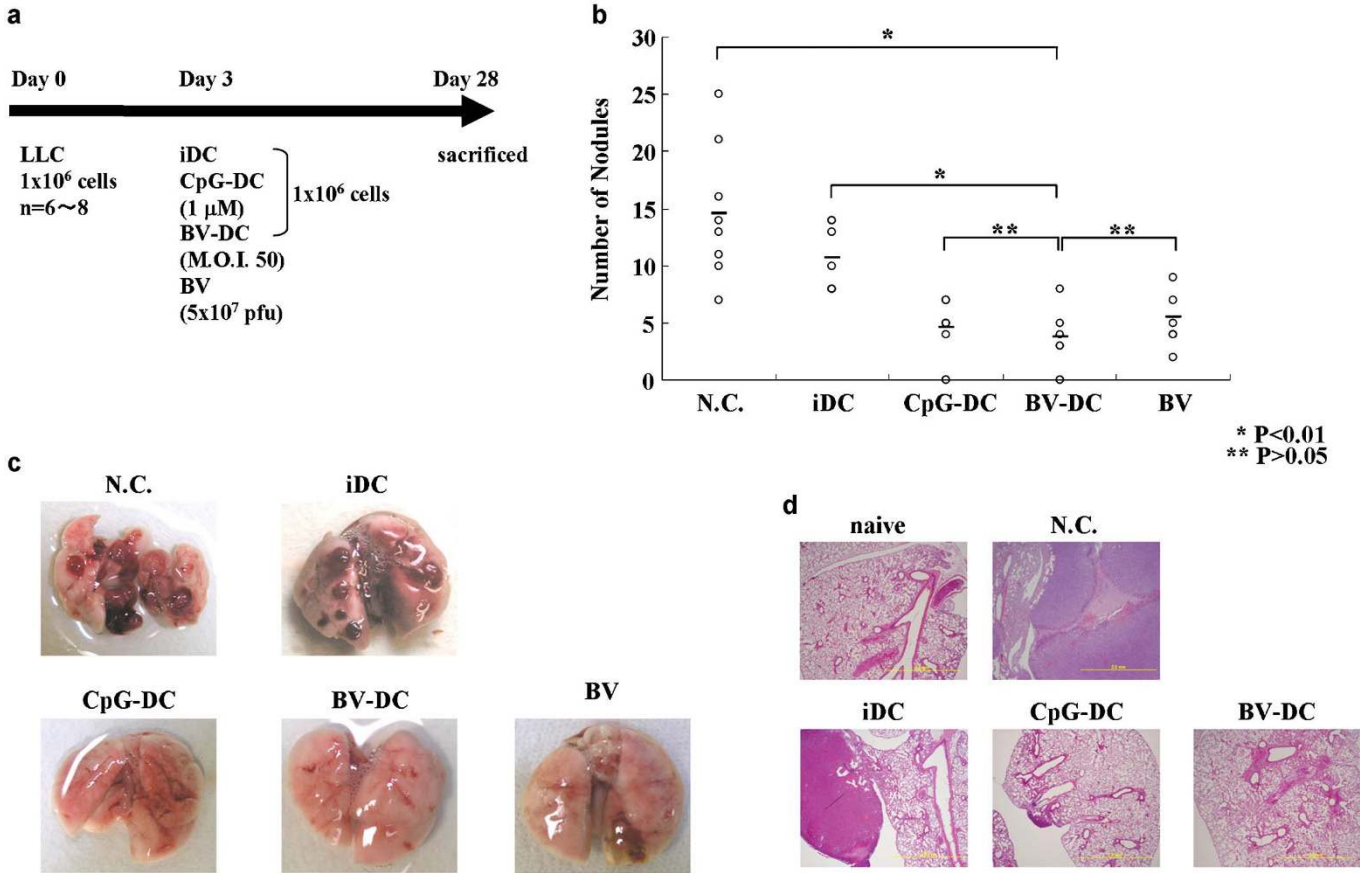

Figure 1 BV-DCs mediate antitumor immunity against LLC lung cancer. LLC cells were intravenously injected into mice (1×10^6 cells/mouse) on day 0, followed by one intravenous injection (1×10^6 cells/mouse) of iDCs, CpG-DCs, BV-DCs or wild-type baculovirus (5×10^7 pfu/mouse) on day 3. (**a**) Experimental design used to assess the antitumor immunity conferred by various DCs. (**b**) Dot plots indicate the number of nodules of LLC following treatment with various DCs or wild-type baculovirus, 28 days after tumor cell inoculation. **P*<0.01, compared with the negative control and iDC treatment. ***P*>0.05, compared with the CpG-DC and wild-type baculovirus treatment. (**c**) Images of lungs excised from tumor-bearing mice 28 days after various DC and wild-type baculovirus treatments. (**d**) Hematoxylin and eosin-stained tissue sections of the excised lungs. Similar results were obtained in two independent experiments. BV, baculovirus; BV-DC, baculovirus-infected bone marrow-derived dendritic cell; CpG-DC, CpG (1 μM)-treated bone marrow-derived dendritic cell; DC, dendritic cell; iDC, immature dendritic cell; LLC, Lewis lung carcinoma; MOI, multiplicity of infection; NC, negative control; pfu, plaque forming unit.

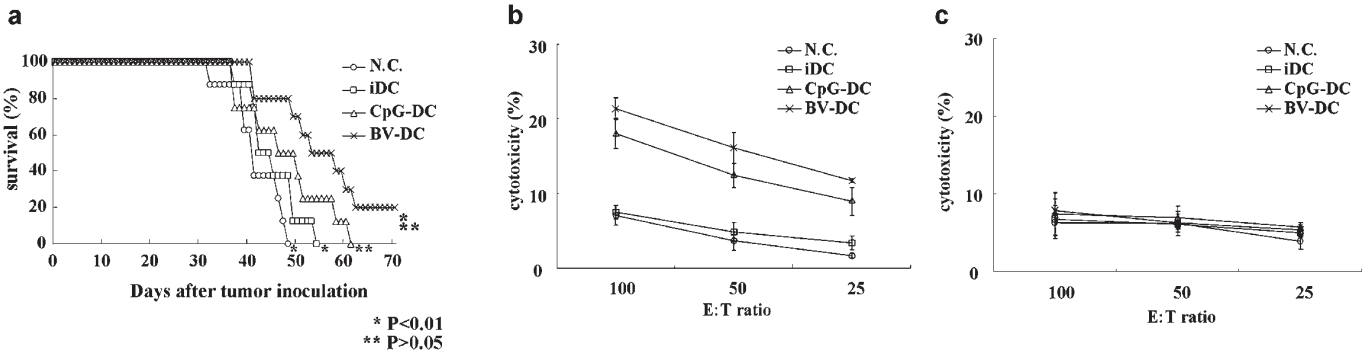

Figure 2 BV-DCs increased the survival of LLC tumor-bearing mice and showed increased cytotoxicity against LLC cells. LLC cells were intravenously injected into mice (1×10^6 cells/mouse) on day 0, followed by one intravenous injection (1×10^6 cells/mouse) of immature iDCs, CpG-DCs or BV-DCs on day 3. (**a**) Survival rate of LLC lung metastasis-bearing mice treated with various DCs. *P<0.01, compared with the negative control and iDC treatment. **P>0.05, compared with the CpG-DC treatment. Splenocytes were isolated from immunized mice 28 days after BMDC immunization, and a cytotoxicity assay was performed. Cytotoxicity against LLC (**b**) or EL4 cells (**c**) by splenocytes from tumor-bearing mice treated with various BMDCs. Similar results were obtained in two independent experiments. BMDC, bone marrow-derived dendritic cell; BV-DC, baculovirus-infected bone marrow-derived dendritic cell; CpG-DC, CpG (1 μM)-treated bone marrow-derived dendritic cell; DC, dendritic cell; iDC, immature dendritic cell; LLC, Lewis lung carcinoma; NC, negative control.

experimental schedule is shown in Figure 3a. Mice were inoculated with B16F10 tumor cells and immunized 10, 17 and 24 days later with iDCs, CpG-DCs or BV-DCs. As shown in Figure 3b and c, BV-DCs inhibited tumor growth as compared with the other control BMDCs. The survival time of the mice immunized with BV-DCs was significantly prolonged as compared with that of the mice inoculated with control BMDCs (Figure 3d). Cytotoxicity was assessed for each group of mice, and BV-DC-inoculated mouse splenocytes demonstrated an upregulation of cytotoxicity against B16F10 cells as compared with the other groups (Figure 4a). EL4 cells were used as target cells in a control experiment, and negative results were obtained (Figure 4b). Furthermore, we investigated the subsets of effector cells that showed cytotoxicity against B16F10 cells by *in vivo* depletion analysis. $CD4^+$ or $CD8^+$ T cell-depleted mice were established by injecting ascites fluid from anti-CD4 or anti-CD8 hybridoma-bearing mice. NK cell-depleted mice were established by injection with anti-asialo GM1. As shown in Figure 4c, the efficiency of tumor inhibition was greatly reduced in $CD8^+$ T cell- and NK cell-depleted mice. However, inhibition of tumor growth was still observed in $CD4^+$ T cell-depleted mice. These results suggest that the antitumor effects of BV-DCs against mouse melanoma, as well as their cytotoxic effector functions, were dependent on $CD8^+$ T cells and NK cells.

Levels of ALT, AST and creatinine were not increased in BV-DC-inoculated mice

The wild-type baculovirus-induced activation of host immunity and the inhibition of tumor growth are shown in Figure 1b;[20,21] however, mice treated with wild-type baculovirus also exhibited increased levels of serum ALT, AST and creatinine. We further investigated whether ALT, AST and creatinine increased in response to the administration of BV-DCs. Mice were injected intravenously with 1×10^6 cells/mouse of BV-DCs or 5×10^7 pfu/mouse of wild-type baculovirus. After 24 h, the sera were collected, and the concentrations of AST, ALT and creatinine were measured (Figure 5). BV-DC administration did not increase serum levels of ALT, AST or creatinine, and the administration of wild-type baculovirus increased only serum creatinine and not ALT and AST. This result is presumably due to the low virus titer (5×10^7 pfu) administered in the present study; other studies employed a higher virus titer ($>1\times10^9$ pfu).[21,34] These results indicate that the administration of BV-DCs did not impair liver and kidney functions in mice.

DISCUSSION

Recent clinical research on cancer immunotherapy performed in cancer patients worldwide[1] has investigated lymphocyte activation therapy, NK cell therapy, biological response modifier therapy, antibody therapy and DC therapy. In particular, research has focused on DC therapy because DCs initiate both innate and antigen-specific immune responses. Various methods are used to activate DCs, including tumor cell lysates, CpG-oligodeoxynucleotide (CpG-ODN), cytokines and viral vectors.[25–29] Several groups have reported that viral vectors, including adenoviral and retroviral vectors, infect DCs and subsequently, infected DCs express tumor antigens and undergo maturation. Tumor growth is inhibited by inoculation of such tumor antigen-expressing DCs in tumor-bearing mice. However, in humans, the presence of neutralizing antibodies against adenovirus has necessitated immunization with a high titer of virus. Retrovirus vectors are considered unsafe because they can integrate into the host genome. Furthermore, immunotherapy with deactivated DCs does not suppress various cancers. Therefore, we examined the requirement for DC activation to induce effective antitumor activity with guaranteed safety in humans.

Baculoviruses have been widely studied as a new viral vector. In particular, studies have reported the development of various antigen-expressing baculoviruses that target specific infections.[11–15] The recombinant baculovirus vectors strongly induced host innate and acquired immunity. We have previously reported that wild-type baculovirus induces antitumor immunity.[21] We also showed that DCs are the immune cells most efficiently infected with baculovirus and that baculovirus-infected DCs trigger the activation of NK cells and T cells.[22]

The current study demonstrated that baculovirus-infected DCs inhibited lung cancer and melanoma in mice. First, we investigated the efficacy of BV-DCs in the inhibition of mouse lung cancer. BMDCs were infected with baculovirus at an MOI of 50. We optimized the virus titer as previously reported.[22] Mice used as a model for lung cancer showed inhibition of tumor growth in response to inoculation with BV-DCs (Figures 1b–d and 2a). Toura *et al.* reported a similar experiment in which they induced antitumor immunity using α-galactosylceramide-activated DCs against LLCs; however, they used fewer tumor cells (4×10^5 cells/mouse) and numerous DCs (3×10^6 cells/mouse) were inoculated.[30] Furthermore, DCs were injected 1 day after

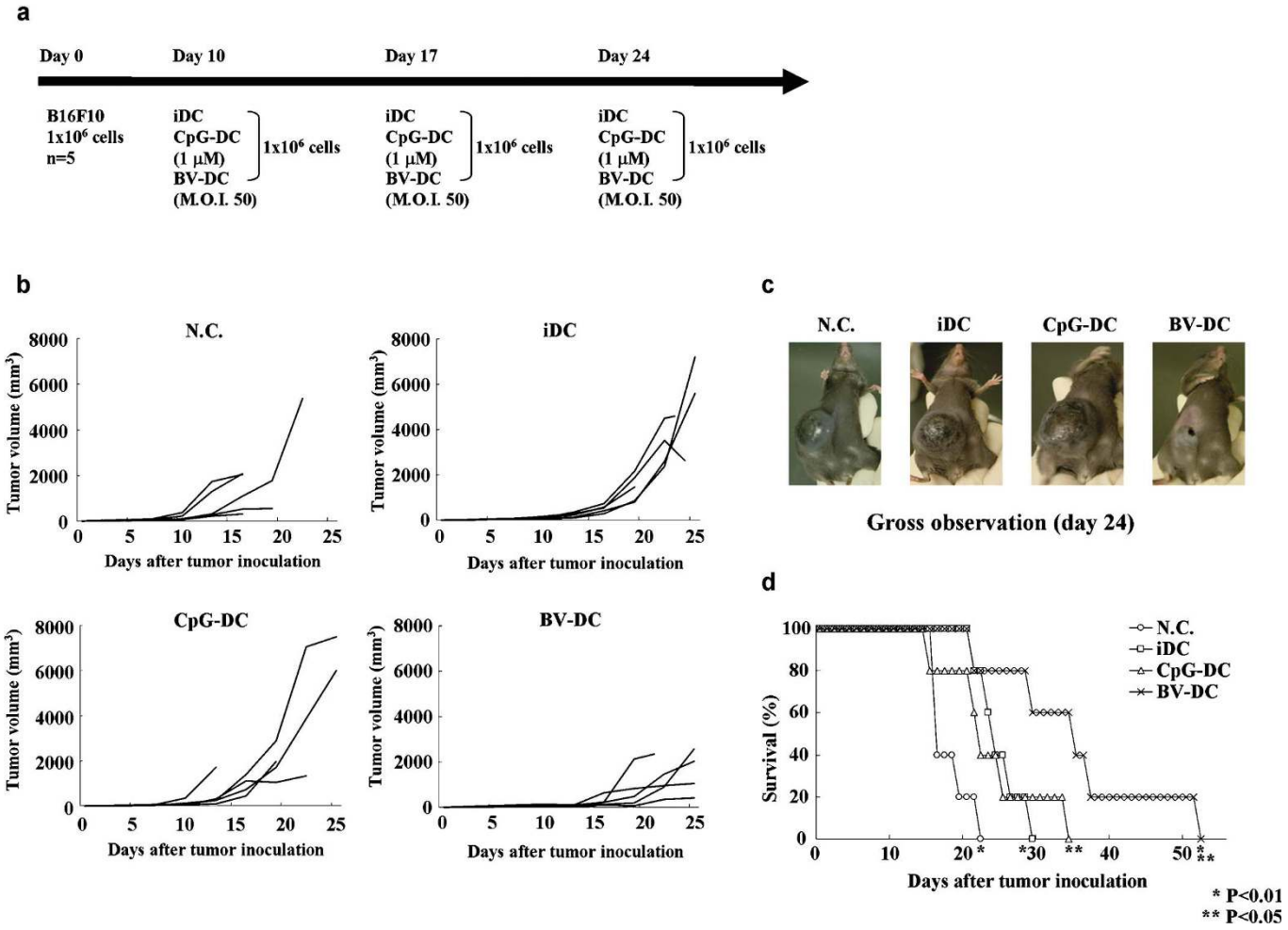

Figure 3 BV-DCs mediate antitumor immunity against melanoma. B16F10 cells were subcutaneously injected into mice (1×10^6 cells/mouse) on day 0, followed by three intratumor injections (1×10^6 cells/mouse) of iDCs, CpG-DCs or BV-DCs on days 10, 17 and 24. Tumor volume was measured every 3 days. (**a**) Experimental design used to assess the antitumor immunity of various DCs. (**b**) Tumor volumes were calculated over time in BMDC-treated mice. Ten days after tumor inoculation, BMDCs were intratumorally injected into each mouse. BMDCs were inoculated three times at weekly intervals. (**c**) Characterization of the established B16F10 tumor. (**d**) Survival rate of B16F10 melanoma tumor-bearing mice treated with various BMDCs. *$P<0.01$, compared with the negative control and iDC treatment. **$P<0.05$, compared with the CpG-DC treatment. Similar results were obtained in two independent experiments. BMDC, bone marrow-derived dendritic cell; BV-DC, baculovirus-infected bone marrow-derived dendritic cell; CpG-DC, CpG (1 μM)-treated bone marrow-derived dendritic cell; DC, dendritic cell; iDC, immature dendritic cell; MOI, multiplicity of infection; NC, negative control.

tumor inoculation. Previous reports showed that wild-type baculovirus could induce innate and acquired immunity,[21,31] and similar results were obtained in the present study using BV-DCs (Figure 2b and c). However, the use of baculovirus in clinical applications for humans is still being analyzed, and there remain some obstacles to overcome, such as safety concerns and optimal dose, before baculovirus can be approved for use in tumor therapy. Here, we examined the antitumor effects of BV-DCs against established tumors in mice. Intratumoral injection of BV-DCs inhibited tumor growth and prolonged survival (Figure 3b–d). Western blot analysis using anti-gp64 mAb to detect baculovirus gp64 in the lysates of BV-DCs at 1 and 3 h post-infection showed the degradation and clearance of baculovirus 3 h post-infection in BMDCs (Supplementary Figure 1). This result indicates that the antitumor activity of BV-DCs is mediated by the BV-DCs and not by the remaining virus. The spleens of immunized mice exhibited increased cytotoxicity against B16F10 cells (Figure 4a). In Figure 4b, EL4 target cells showed a negative result. Our results demonstrate that CpG-DCs could inhibit LLC, but not melanoma, whereas BV-DCs inhibited the growth of both tumor types. Therefore, we considered concluded that the inhibition of tumor growth by CpG-DCs is tumor cell type-dependent. To analyze the involvement of specific immune cell types in this tumor inhibition, $CD4^+$ and $CD8^+$ T cell- and NK cell-depleted mice were produced by injecting mice intraperitoneally with specific neutralizing antibodies. BV-DCs were inoculated into $CD4^+$ and $CD8^+$ T cell- and NK cell-depleted tumor-bearing mice. $CD8^+$ T cell- or NK cell-depleted mice showed a reversed effect on the inhibition of tumor growth (Figure 4c). We therefore speculated that antitumor immunity is dependent on $CD8^+$ T cells or NK cells but not on $CD4^+$ T-cell activity. Several groups have reported that regulatory T cells ($CD4^+CD25^+Foxp3^+$) are induced by tumor cells and inhibit antitumor immunity, whereas other groups have reported an inhibitory effect of tumor cells that suppresses the host antitumor immune response.[32–34] Our data suggest that regulatory T cells may inhibit tumor growth suppression. Previously, we and other groups reported that wild-type baculovirus impairs liver function in mice.[21,35] As shown in Figure 5, BV-DCs did

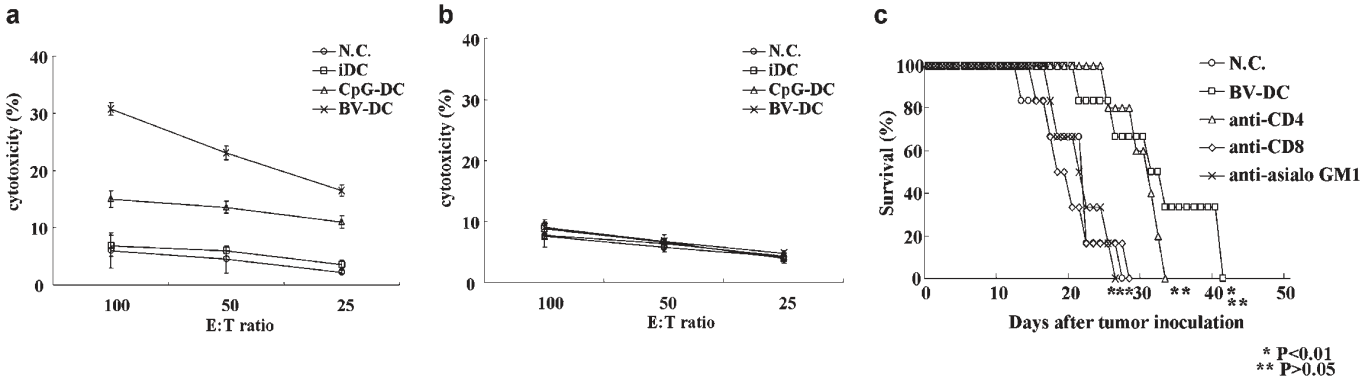

Figure 4 BV-DCs show increased cytotoxicity against B16F10 melanoma cells and depletion of $CD8^+$ T cells or NK cells leads to decreased survival of immunized mice. B16F10 cells were subcutaneously injected into mice (1×10^6 cells/mouse) on day 0, followed by three intratumoral injections (1×10^6 cells/mouse) of iDCs, CpG-DCs or BV-DCs on days 10, 17 and 24. Splenocytes were isolated from immunized mice 7 days after the first immunization with BMDCs, and a cytotoxicity assay was performed. Cytotoxicity against B16F10 (**a**) or EL4 cells (**b**) by splenocytes from tumor-bearing mice treated with various BMDCs. (**c**) Depletion of $CD4^+$, $CD8^+$ or NK cells by treatment with specific antibodies. Tumor-inoculated mice were intraperitoneally injected with ascites from hybridoma-bearing mice or anti-asialo GM1. The experimental schedule is described in the section on 'Materials and methods'. **P*<0.01, compared with the negative control, anti-CD8 antibody-treated mice and anti-asialo GM1-treated mice. ***P*>0.05, compared with anti-CD4 antibody-treated mice. Similar results were obtained in two independent experiments. BMDC, bone marrow-derived dendritic cell; BV-DC, baculovirus-infected bone marrow-derived dendritic cell; CpG-DC, CpG (1 μM)-treated bone marrow-derived dendritic cell; iDC, immature dendritic cell; NC, negative control; NK, natural killer.

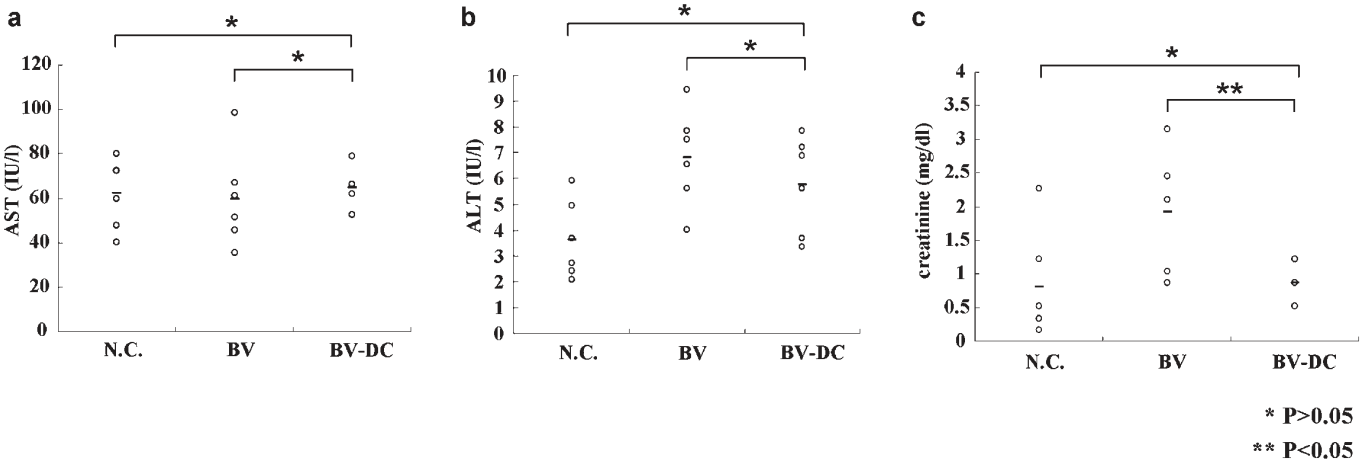

Figure 5 Serum AST, ALT and creatinine levels in BV-DC-immunized mice. Wild-type baculovirus or BV-DC was intravenously inoculated into mice. The sera were collected after 24 h, and AST (**a**), ALT (**b**) and creatinine (**c**) levels were measured. **P*>0.05, compared with the negative control and wild-type baculovirus treatment. ***P*<0.05, compared with wild-type baculovirus treatment. Similar results were obtained in two independent experiments. BV, baculovirus; BV-DC, baculovirus-infected bone marrow-derived dendritic cell; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

not cause any impairment of mouse liver or kidney functions; therefore, we consider BV-DCs to be safer and more effective than wild-type baculovirus.

In summary, we showed that BV-DCs induced antitumor immunity against established tumors in mice. These antitumor effects were $CD8^+$ T cell- and NK cell-dependent but $CD4^+$ T cell-independent. Therefore, BV-DCs might be a useful tool for immunotherapy against malignancies.

Note: Supplementary information is available on the Cellular & Molecular Immunology website (http://www.nature.com/cmi/).

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