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Anti-Tumor Activity of *Acinetobacter baumannii* Outer Membrane Protein A on Dendritic Cell-Based Immunotherapy against Murine Melanoma

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***Acinetobacter baumannii* outer membrane protein A (AbOmpA) is a major surface protein that is an important pathogen-associated molecular pattern. Based on our previous findings that AbOmpA induced the phenotypic maturation of dendritic cells (DCs) and drove the Th1 immune response *in vitro*, we investigated the therapeutic efficacy of AbOmpA-pulsed DC vaccines in a murine melanoma model. The surface expression of co-stimulatory molecules (CD80 and CD86) and major histocompatibility complex class I and II molecules was higher in DCs pulsed with AbOmpA alone or with a combination of B16F10 cell lysates than that of DCs pulsed with B16F10 cell lysates. AbOmpA stimulated the maturation of murine splenic DCs *in vivo*. In a therapeutic model of murine melanoma, AbOmpA-pulsed DCs significantly retarded tumor growth and improved the survival of tumor-bearing mice. AbOmpA-pulsed DCs significantly enhanced $CD8^+$, interleukin-2^+ T cells and $CD4^+$, interferon-γ^+ T cells in tumor-bearing mice. These results provide evidence that AbOmpA may be therapeutically useful in adjuvant DC immunotherapy against poorly immunogenic melanoma without tumor-specific antigens.**

***Keywords*:** dendritic cells, outer membrane protein, melanoma, vaccine, cytokine

Dendritic cells (DCs) are an attractive vector for cancer immunotherapy because of their unique properties. They have high antigen capturing and presenting capacity, which can result in the efficient induction and maintenance of immune responses (Banchereau *et al.*, 2000). DCs are capable of stimulating naïve T cells and producing immunomodulatory cytokines, which in turn regulate multiple effector cells, including $CD8^+$ cytotoxic T lymphocytes (CTLs), natural killer (NK) cells, B cells, monocytes, and macrophages. These cells can serve to induce anti-tumor immune responses. Tumor-associated antigen (TAA)-loaded DCs are able to activate tumor-specific T cells and induce anti-tumor immunity in animal models and some clinical trials (Fields *et al.*, 1998). Several antigen delivery systems, including the pulsing of DCs with defined tumor peptides or whole cell lysates (Sallusto *et al*., 1995), viral vector systems transfected with defined tumor peptides (Shibagaki and Udey, 2002), and the fusion of DCs with tumor cells (Parkhurst *et al.*, 2003), have been employed to achieve optimally-matured DCs. Moreover, immunoadjuvants combined with TAA are used for DC-pulsing in order to induce strong anti-tumor immunity (Chagnon *et al*., 2005).

Malignant melanoma causes significant health problems because of its morbidity and mortality. The incidence of melanoma in Caucasian populations has been substantially rising for several decades, whereas it is relatively uncommon in Asian countries (Brerwick and Halpern, 1997; Garbe and Blum, 2001). Although the primary intervention of cutaneous melanoma is surgical excision, adjuvant treatment modalities, such as chemotherapy, radiotherapy, non-specific immunotherapy, and interferon (IFN) treatment, have been investigated in high-risk melanoma patients with metastatic diseases or with tumors >1.5 mm in thickness (Garbe and Eigentler, 2007). Among the adjuvant melanoma therapies, only IFN-α treatment was approved by the Food and Drug Administration in the USA and European authorities. None of the clinical trials with Bacillus Calmette-Guérin or other non-specific immunostimulatory strategies showed any benefit to melanoma patients (Mohr *et al*., 2003; Verma *et al*., 2006). Recently, DC-based immunotherapy has been clinically approved and it has been therapeutically tried in the treatment of melanoma (O'Rourke *et al*., 2007).

Bacterial components, such as lipopolysaccharides, peptidoglycans, and unmethylated cytosine-phosphorothionate-guanine-rich oligodeoxynucleotides (CpG-ODN), are able to activate immune responses through the binding of toll-like receptors (TLRs) (Tsan and Baochong, 2007). Outer membrane proteins (Omps) of Gram-negative bacteria play a significant role in bacterial pathogenesis and they also have potent immunostimulatory effects. PorA and Omp complex from *Neisseria meningitidis* activate human monocyte-derived DCs through TLR2 (Al-Bader *et al*., 2004). Conjugative vaccines consisting of the capsular polysaccharides of *Haemophilus influenzae* type b (Hib), that are covalently linked to the Omp complex from *N. meningitidis*, have been successfully developed for Hib vaccine (Weinberg

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et al., 1987; Latz *et al*., 2004). More interestingly, OmpA of *Klebsiella pneumoniae* (kpOmpA) induces the maturation of DCs through TLR2 and therapeutic immunities are elicited to antigen-expressing tumors (Jeannin *et al.*, 2000). We have demonstrated that OmpA of *Acinetobacter baumannii* (AbOmpA) induced the maturation of DCs through TLR2 and it drove the Th1 polarization of immune responses *in vitro* (Lee *et al*., 2007). These findings implicate that AbOmpA has potential as an immunoadjuvant toward anti-infectious and anti-tumor vaccines. In the present study, we investigated the anti-tumor activity of AbOmpA on DC-based immunotherapy against murine melanoma in a therapeutic setting.

Materials and Methods

Animals and tumor cells

C57BL/6 male mice (6∼8 weeks old) were purchased from the Korean Institute of Chemistry Technology (Korea) and housed in an animal facility for at least one week before use. All procedures involving animals were approved by the Animal Care Committee of Kyungpook National University School of Medicine. The poorly immunogenic B16F10 melanoma cells, a highly malignant subline of the B16 melanoma, were obtained from Korean Cell Line Bank. B16F10 melanoma cells were cultured in Dulbecco's modified Eagle's medium supplemented with 2 mM L-glutamine, 1,000 U/ml penicillin, 50 μg/ml streptomycin, and 10% fetal bovine serum (FBS). Cells were maintained at 37°C in 5% CO_2. The confluent growth was obtained in 100 mm diameter dishes and cells were routinely passaged every three days.

Reagents and antibodies

Recombinant mouse granulocyte-macrophage colony stimulating factor (GM-CSF) and interleukin (IL)-4 were purchased from R&D Systems. FITC- or phycoerythrin-conjugated monoclonal antibodies were used to detect the expression of CD4 (L3T4), CD8 (Ly-2), CD11c (HL3), CD40 (1C10), CD80 (16-10A1), CD86 (GL1), major histocompatibility complex (MHC) class I (H-$2K^b$), and MHC class II (I-A^b). The intracellular expression of IL-2 (JES6-5H4) and IFN-γ (XMG1.2) was measured by flow cytometry. The isotype-matched control monoclonal antibody, biotinylated anti-D11c (N418), was used. All reagents were purchased from BD Pharmingen.

Preparation of AbOmpA and cell lysates

The *ompA* gene from *A. baumannii* ATCC 19606^T was cloned in the pET28a expression vector. *E. coli* BL21 (DE3)/pET28a harboring the *ompA* gene was grown in a Luria-Bertani medium at 37°C and the recombinant AbOmpA proteins were overexpressed with 1 mM of IPTG at 25°C for 4 h. The purification of AbOmpA and the removal of endotoxins were performed as previously described (Lee *et al*., 2007). Tumor cell lysates were prepared by four cycles of a freezing and thawing of B16F10 melanoma cells. Cellular debris was removed by low speed centrifugation and the soluble fraction was passed through a 0.22 μm membrane filter. The protein concentration was adjusted to 10 μg/ml and stored at -70°C.

Generation of bone marrow-derived DCs and antigen pulsing

DCs were generated from murine bone marrow-derived cells with some modifications, as previously described (Winzler *et al*., 1997). Erythrocyte-depleted bone marrow-derived cells from the tibia and femur of C57BL/6 mice were cultured in OptiMEM (Invitrogen Life Technologies, USA) supplemented with 10% FBS, 20 ng/ml recombinant mouse GM-CSF, and 20 ng/ml IL-4 at 37°C in an atmosphere of 5% CO_2. On day 3 of culturing, floating cells were removed and fresh medium containing GM-CSF and IL-4 was replaced. On days 6∼7 of culturing, non-adherent cells and loosely adherent proliferating DC aggregates were harvested. More than 80% cells expressed CD11c were used in the further experiments. For the antigen pulsing, B16F10 cells were collected using trypsin/EDTA solution (Life Technologies Inc., USA), washed with a phosphate-buffered saline (PBS), and resuspended in a balanced salt solution (10 mM Tris-HCl, 150 mM NaCl, pH 7.6). DCs (1×10^6 cells/ml) were pulsed with either 10 μg/ml of B16F10 cell lysates, 200 ng/ml of AbOmpA or both antigens at 37°C for 18 h.

Animal experiments

B16F10 melanoma cells (2×10^5) were suspended in a 100 μl of PBS and the cells were injected subcutaneously into the right lower back of mice. DCs (1×10^6) pulsed with antigens or unstimulated immature DCs were injected subcutaneously into the opposite side of back according to the time schedules. Each group of tumor-bearing mice was treated with one of following: a PBS, unstimulated immature DCs, DCs pulsed with B16F10 cell lysates, DCs pulsed with AbOmpA, or DCs pulsed with B16F10 cell lysates and AbOmpA. Tumors were measured at intervals with 2∼5 days and tumor mass was calculated as follows: $V = (A^2\times B)/2$, where A is the length of the short axis and B is the length of the long axis.

Flow cytometric analysis

Single cell suspensions of DCs or lymphocytes were stained with specific monoclonal antibodies. To prepare splenic DCs and lymphocytes, mice were euthanized by CO_2 inhalation. The spleens were mechanically disrupted and passed through a sterile nylon mesh filter (BD Falcon, USA). The resulting cells were centrifuged at 400×g for 5 min and single cell suspensions were prepared. DCs were analyzed using flow cytometry (FACSCalibur, Becton Dickinson, USA) with gating on $CD11c^+$ cells. Lymphocytes were analyzed using flow cytometry with gating on $CD4^+$ T or $CD8^+$ T cells. Intracellular IL-2 and IFN-γ were stained with fluorescein R-phycoerythrin-conjugated antibodies in a permeation buffer. Cells were fixed and permeated by a Cytofix/Cytoperm kit (BD Pharmingen, USA) according to manufacturer's instructions.

Statistical analysis

Statistical analyses were performed using SPSS (SPSS Inc., USA). Differences were compared with regard to statistical significance by a one-way ANOVA. Log rank test was performed to compare survival rates of the mice. Values of $P<0.05$ were considered to be statistically significant.

Results

AbOmpA induces the maturation of bone marrow-derived DCs *in vitro* and splenic DCs *in vivo*

We previously demonstrated that ≤200 ng/ml of AbOmpA induced the maturation of murine bone marrow-derived DCs (Lee *et al*., 2007). In order to compare the maturation of DCs, DCs were pulsed with B16F10 cell lysates, AbOmpA, or the combination of B16F10 cell lysates and AbOmpA. DCs pulsed with antigens were analyzed by flow cytometry for the expression of cell surface molecules involved in T cell activation. Antigen-pulsed DCs exhibited an increased expression of surface markers, B7 family (CD80 and CD86) and MHC class I and II molecules compared with the untreated immature DCs (Fig. 1). The expression of CD80 and CD86 molecules was the highest in DCs pulsed with a combination of B16F10 cell lysates and AbOmpA, whereas the expression of MHC class I and II molecules was the highest in DCs pulsed with AbOmpA. The expression of surface markers tested was the lowest in DCs pulsed with B16F10 cell lysates, suggesting that B16F10 melanoma is a poorly immunogenic tumor.

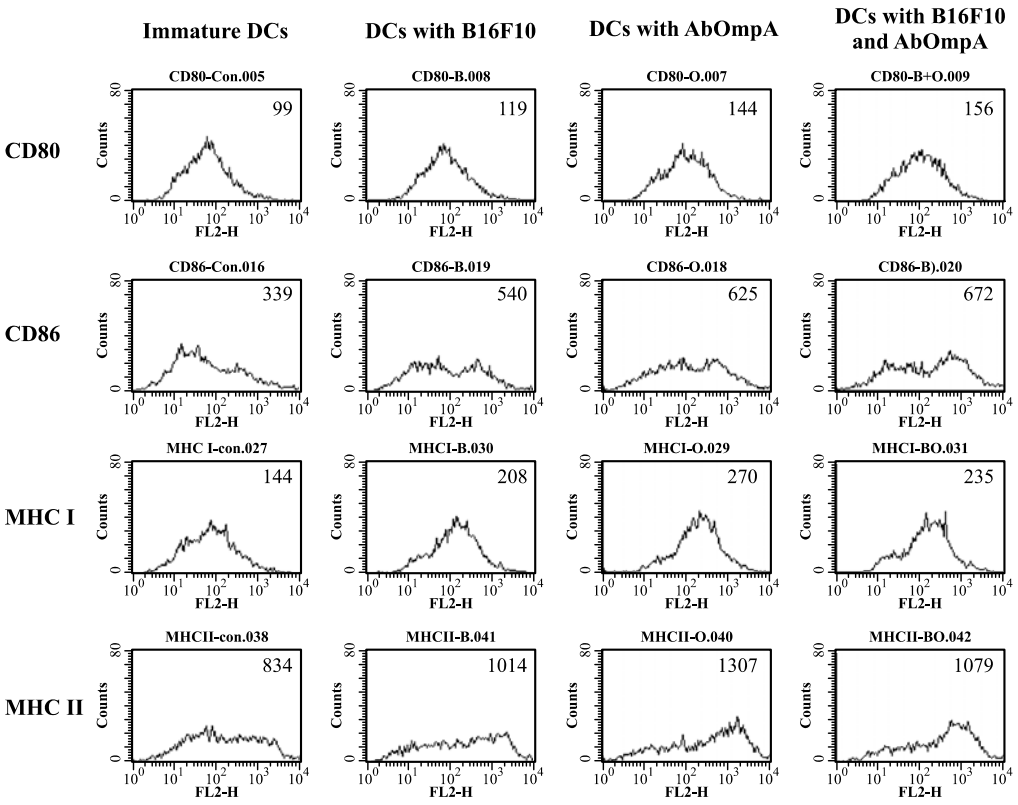

Fig. 1. Phenotypic maturation of DCs pulsed with different antigens. DCs were pulsed with AbOmpA (200 ng/ml), B16F10 cell lysates (10 μg/ml), and both antigens for 18 h. Cells were harvested and analyzed by two-color flow cytometry. Cells were first gated on $CD11c^+$ and the expressions of CD80, CD86, and MHC class I and II molecules were analyzed. The values are the mean fluorescence intensity. The data are representative of three experiments that gave similar results.

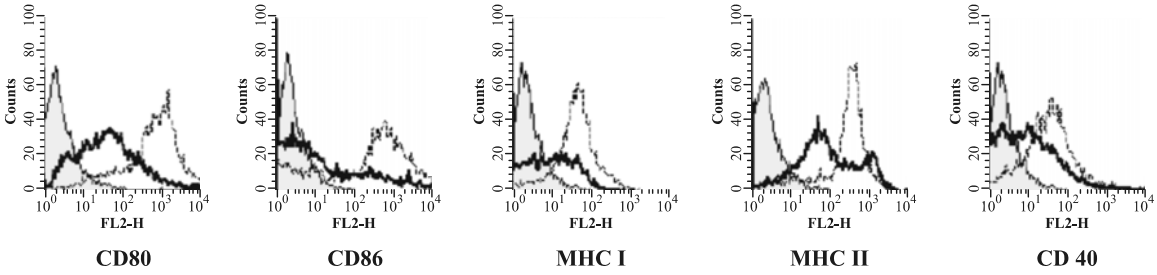

Fig. 2. The maturation of splenic DCs after the AbOmpA challenge. Mice were injected with AbOmpA (1 mg/kg) and splenic DCs were generated two days after the AbOmpA challenge. DCs were analyzed using two-color flow cytometry. Gray filled histogram, black line, and dotted line indicate isotype control, DCs from the untreated control mice, and DCs from the AbOmpA-challenged mice, respectively. The data are representative of two independent experiments that gave similar results.

To determine whether AbOmpA induces the activation and maturation of DCs *in vivo*, AbOmpA (1 mg/kg) was injected intraperitoneally and the expression of surface markers on the splenic DCs was analyzed. The expression of surface markers, CD40, CD80, CD86, and MHC class I and II molecules, on the splenic DCs increased two days after the AbOmpA challenge, as compared with that of the splenic DCs from the untreated control mice (Fig. 2). These results suggest that AbOmpA is a potent immunoadjuvant for the DC maturation *in vitro* and *in vivo*.

AbOmpA-pulsed DCs retard tumor growth and improve the survival of tumor-bearing mice

We determined whether DCs pulsed with AbOmpA alone or in combination with autologous tumor cell lysates elicited therapeutic anti-tumor immunity against low tumor burden. Groups of 10 mice were injected subcutaneously with B16F10 cells (2×10^5) on day 0 and then the mice were treated three times with a PBS, unstimulated immature DCs, DCs pulsed with B16F10 cell lysates, DCs pulsed with AbOmpA, and DCs pulsed with a combination of B16F10 cell lysates and AbOmpA on days 1, 7, and 13 (Fig. 3). By day 12 post-tumor injection, the tumors in mice that received unstimulated immature DCs or DCs pulsed with antigens were significantly smaller than those in the mice treated with a PBS ($P<0.001$), but tumor mass was not significantly different between the mice groups treated with DCs. On day 17, the mice treated with AbOmpA-based DCs had developed small tumors, with a size of 356.0±203.4 mm^3 in DCs pulsed with AbOmpA and 760.5±650.6 mm^3 in DCs pulsed with B16F10 cell lysates and AbOmpA, whereas the tumors in mice treated with DCs pulsed with B16F10 cell lysates were 1857.4±734.5 mm^3. Tumors rapidly grew in all groups of mice 17 days after the tumor challenge. The control mice that were treated with a PBS died between day 18 and 23. On day 25 post-tumor injection, the tumors in mice treated with AbOmpA-based DCs were significantly smaller as compared to those in mice treated with unstimulated immature DCs or DCs pulsed with B16F10 cell lysates ($P<0.005$). These results suggest that DCs pulsed with AbOmpA alone or in a combination with autologous tumor cell lysates significantly retarded tumor growth. However, the survival rates of each group were not significantly different on day 25 post tumor injection. The following number of mice died in the groups

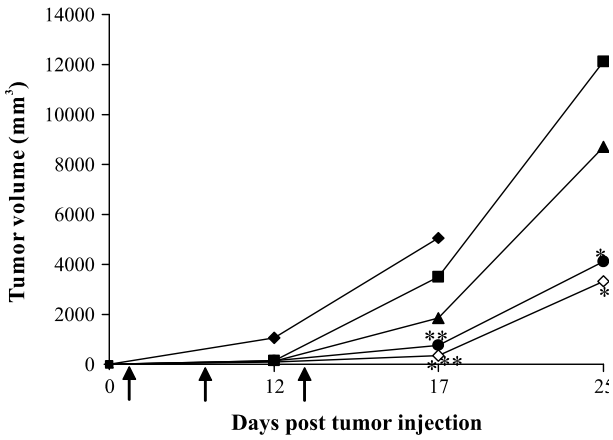

Fig. 3. Therapeutic anti-tumor activity of AbOmpA-based DC vaccines against early tumor burden. A group of 10 mice were injected with B16F10 cells (2×10^5) on the day 0. The mice were treated with a DC vaccine on days 1, 7, 13 after the tumor injection. Mice were treated with either a PBS (◆), unstimulated immature DCs (■), DCs pulsed with B16F10 cell lysates (▲), DCs pulsed with AbOmpA (◇), and DCs pulsed with the combination of B16F10 cell lysates and AbOmpA (●). The tumor size was measured on days 12, 17, and 25 after the tumor injection. Arrows represent DC vaccine challenges and points are a mean of 10 mice. (*) $P<0.005$ and (**) $P<0.001$, statistically significant smaller tumors when comparing AbOmpA-pulsed DCs and DCs pulsed with AbOmpA and B16F10 cell lysates to unstimulated DCs.

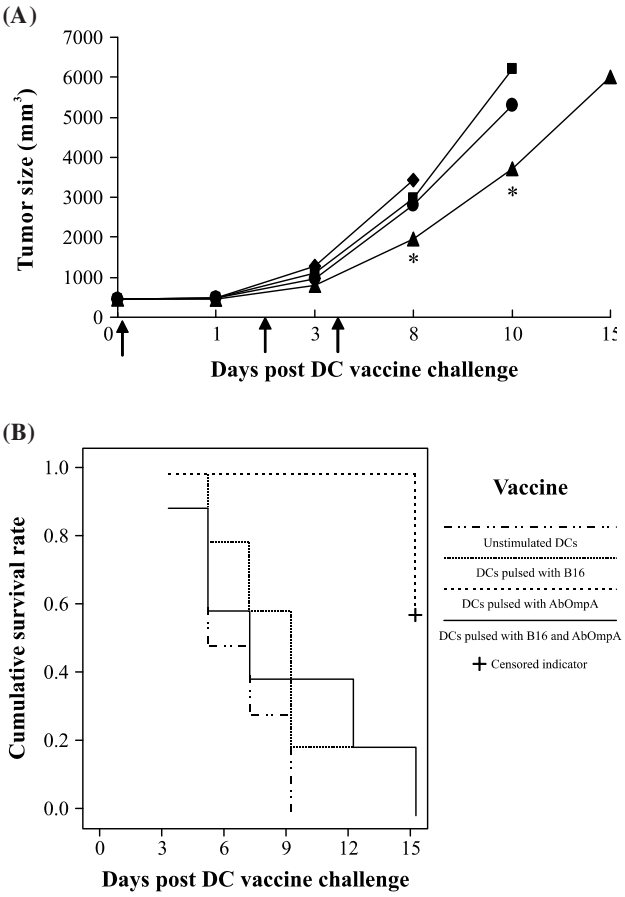

Fig. 4. Therapeutic anti-tumor activity of AbOmpA-pulsed DC vaccines against established tumors. Mice were injected with B16F10 cells (2×10^5) and the tumors grew to 450~500 mm^3 in size. The tumor-bearing mice were separated into four groups: treatment with unstimulated immature DCs (◆), B16F10 cell lysate-pulsed DCs (■), AbOmpA-pulsed DCs (▲), and B16F10 cell lysates and AbOmpA-pulsed DCs (●). DC vaccines were injected into the opposite side of back three times at two days interval. Arrows indicate a challenge of DC vaccines. (A) The tumors were measured on days 1, 3, 8, 10, and 15 after the first DC vaccine challenge. The points are the mean tumor volume. (*) $P<0.05$, statistically significant smaller tumors when comparing AbOmpA-pulsed DCs to unstimulated DCs or DCs pulsed with other antigens. (B) Survival rates are the percentage of the surviving mice from a total of 10 mice per group.

of unstimulated immature DCs (n=3), B16F10 cell lysate-pulsed DCs (n=2), B16F10 cell lysates and AbOmpA-pulsed DCs (n=2), and AbOmpA-pulsed DCs (n=1).

To determine whether AbOmpA-based DCs induce therapeutic anti-tumor immunity against established tumors, B16F10 cells (2×10^5) were injected into the back of mice and tumors were allowed to grow up to 450~500 mm^3. Tumor-bearing mice were separated into four groups: the mice were to be treated with unstimulated immature DCs, B16F10 cell lysate-pulsed DCs, AbOmpA-pulsed DCs, and B16F10 cell lysate and AbOmpA-pulsed DCs. Each DC vaccine was injected into the opposite side of back three times at two days interval. On day 3 after the first DC vaccination, the tumor sizes were not significantly different between four groups (Fig. 4A). However, tumor growth was significantly retarded in the mice treated with DCs pulsed with AbOmpA, as compared to the other groups 8 days after the first DC vaccination ($P<0.05$). The mice treated with unstimulated immature DCs, DCs pulsed with B16F10 cell lysates, and DCs pulsed with B16F10 cell lysates and AbOmpA all died 15 days after the first DC vaccination, whereas 60% of the mice treated with DCs pulsed with AbOmpA survived over the same period of time (Fig. 4B). These data suggest that AbOmpA-pulsed DCs retard tumor growth and improve the survival of tumor-bearing mice in a murine melanoma model.

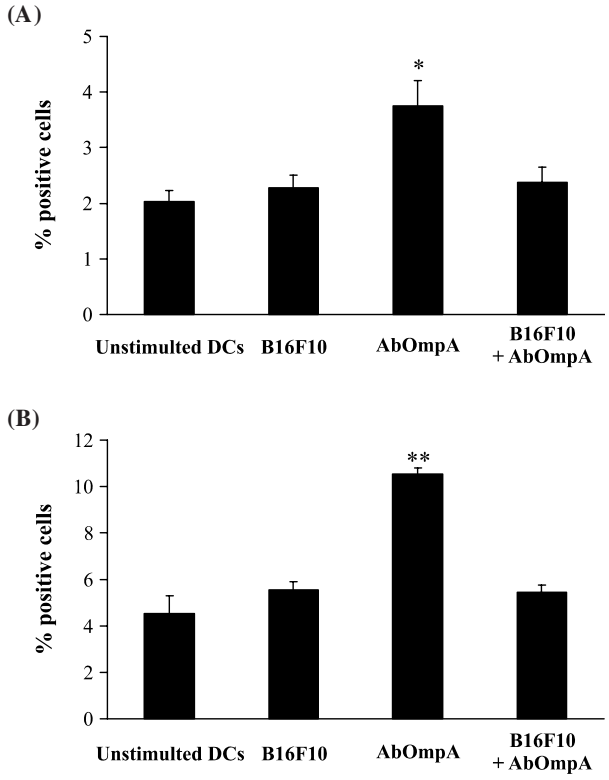

Fig. 5. The functional changes of splenic T cells in tumor-bearing mice treated with antigen-pulsed DCs. Groups of three mice were injected with B16F10 cells subcutaneously into the back of mice and were allowed to grow up to sizes of 700~800 mm^3. Established tumors were treated with antigen-pulsed DCs three times at two days interval. Lymphocytes were generated from the spleens two days after the last vaccination. The $CD8^+IL\text{-}2^+$ T cells (A) and $CD4^+$ IFN-γ^+ T cells (B) were counted by flow cytometry. (*) $P<0.05$ and (**) $P<0.001$, statistically significant increase of $CD8^+IL\text{-}2^+$ T cells or $CD4^+$ IFN-γ^+ T cells when comparing AbOmpA-pulsed DCs to unstimulated DCs or DCs pulsed with other antigens.

AbOmpA-pulsed DCs increase $CD8^+IL\text{-}2^+$ and $CD4^+$ IFN-γ^+ T cell populations in tumor-bearing mice

To determine whether the retardation of tumor growth or the improvement of survival in the tumor-bearing mice treated with AbOmpA-pulsed DC vaccine are associated with CTL activity and Th1 immunity in the tumor-bearing host, $CD8^+IL\text{-}2^+$ T cells for the CTL activity and $CD4^+$ IFN-γ^+ T cells for the Th1 immune response were analyzed. IL-2 is an important growth and activation factor for CTLs and typical Th1 cells produce a large quantity of IFN-γ. Groups of three mice were injected with B16F10 cells subcutaneously into the back of mice and tumors were allowed to grow up to sizes of 700~800 mm^3. Antigen-pulsed DCs were injected subcutaneously into the opposite side of back three times at two days interval and the splenic lymphocytes were prepared two days after the last DC vaccination. The number of $CD4^+$IFN-γ^+ T cells and $CD8^+IL\text{-}2^+$ T cells in the mice received AbOmpA-pulsed DCs was significantly different from the value of $CD4^+$IFN-γ^+ T cells and $CD8^+IL\text{-}2^+$ T cells in the mice received unstimulated DCs or DCs pulsed with other antigens (Fig. 5).

Discussion

DC vaccination against tumors is a new immunotherapeutic approach and a number of studies have evaluated anti-tumor efficacy against various types of tumors (O'Neill *et al*., 2004; Banchereau and Palucka, 2005). To develop efficient anti-tumor immunity, DCs present tumor-specific antigens via MHC class I and II molecules and specific immune responses are elicited via $CD8^+$ and $CD4^+$ T cells, respectively. We examined the effects of AbOmpA or in combination with autologous tumor cell lysates on DC-based immunotherapy against a murine B16 melanoma in a therapeutic setting. Based on the previous studies that DC vaccines pulsed with a combination of autologous tumor antigens and immunoadjuvant CpG-ODN induced anti-tumor immunity (O'Neill *et al*., 2004; Chagnon *et al*., 2005), we expected that DCs pulsed with a combination of B16F10 cell lysates and AbOmpA induced a potent anti-tumor immunity against a murine melanoma, but DCs pulsed with AbOmpA alone induced the most potent anti-tumor immunity against both low tumor burden and established tumors, even with the absence of immunologic stimuli that are associated with autologous tumor antigens.

The retardation of tumor growth was correlated with the functional changes of splenic T cells, such as an increase of $CD4^+$IFN-γ^+ and $CD8^+IL\text{-}2^+$ T cells in tumor-bearing mice. Among the antigens used in DC-pulsing, AbOmpA alone was the most potent stimulus for the expression of MHC class I and II molecules, whereas the expression of B7 family (CD80 and CD86) was the highest in DCs pulsed with B16F10 cell lysates and AbOmpA. This inconsistency among the DC maturation markers is possibly due to the interfer-

ence of antigen presentation on the MHC molecules, although both antigens activated DCs through the pattern recognition receptors on cell surface. A high expression of MHC class I and II molecules was also observed in splenic DCs from mice injected with AbOmpA *in vivo*. This result suggests that AbOmpA could be delivered to the MHC class I pathway and primed antigen-specific $CD8^+$ T cells. Jeannin *et al*. (2000) reported that kpOmpA elicited direct CTL responses to exogenous antigens through the cross presentation of kpOmpA to MHC class I molecules. kpOmpA-ovalbumin (OVA) stimulated therapeutic anti-tumor immunity against E.G7 tumor, which is insensitive to NK-mediated lysis and to anti-OVA-mediated complement-dependent cytolysis.

In addition to the direct activation of naïve $CD8^+$ T cells via the antigen-MHC class I complex on DCs, the activation of naïve $CD4^+$ T cells and the subsequent polarization of $CD4^+$ Th1 cells are important for anti-tumor immunity, because $CD4^+$ Th1 cells can cross-prime CTLs and activate various types of effector cells through cytokines. In the current study, AbOmpA-pulsed DCs induced the polarization of Th1 immune responses *in vivo*, as demonstrated by a significant increase of the splenic $CD4^+$IFN-γ^+ T cells from tumor-bearing mice. Our data suggest that the ability of AbOmpA to elicit CTL activation and the polarization of Th1 immune responses can elicit a polyclonal immune response, thereby reducing the possibility of tumors escaping from immune surveillance. The potent therapeutic effect of AbOmpA-pulsed DCs, other than DCs pulsed with autologous cell lysates or in combination with AbOmpA, could be partly explained by the potential of AbOmpA in activating specific anti-tumor immune effector cells.

DCs pulsed with a combination of tumor-specific antigens and CpG-ODN induced effective anti-tumor immunity against murine melanoma (Pilon-Thomas *et al*., 2006). Morevover, Chagnon *et al.* (2005) demonstrated that DCs pulsed with CpG-ODN alone retarded tumor growth and improved the survival of animals in a murine renal cell carcinoma model. An immunoadjuvant, CpG-ODN, induces the maturation of DCs and subsequently secretion of various immunostimulatory cytokines, such as IL-12, IL-18, and IFN-γ. This can create a Th1-like milieu and it may play an integral role in anti-tumor immunity (Krieg, 2002). This finding suggests that the functionally active DCs are essential to induce anti-tumor activity, since tumors can suppress DC function (Gabrilovich *et al*., 1996; Vicari *et al*., 2002; Furumoto *et al*., 2004). Furthermore, CpG-ODN matured and tumor antigen-pulsed DCs did not significantly retard tumor growth (Pilon-Thomas *et al*., 2006), suggesting that the differences in antigen-pulsing and the surface expression of peptides on DCs can affect anti-tumor activity. In the current study, we showed that the fully matured DCs pulsed with a highly immunogenic exogenous antigen AbOmpA could induce therapeutic anti-tumor immunity against the poorly immunogenic murine tumor, in the same manner as DCs pulsed with autologous antigens.

In conclusion, the AbOmpA-based DCs are more potent than DCs pulsed with autologous tumor cell lysates against poorly immunogenic murine melanoma. Although we were unable to define the exact mechanism by which AbOmpA induces strong anti-tumor immunity against murine melanoma, AbOmpA plays a significant role in inducing anti-tumor immune responses in tumor-bearing hosts. This finding implicates that AbOmpA can be applied to DC-based immunotherapy against poorly immunogenic tumors without tumor-specific antigens.

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