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The critical role of Th1-dominant immunity in tumor immunology

Abstract To investigate the precise role of antigen-specific Th1 and Th2 cells in tumor immunity, we developed a novel adoptive tumor-immunotherapy model using OVA-specific Th1 and Th2 cells and an OVA gene-transfected tumor. This therapeutic model demonstrated that both antigen-specific Th1 and Th2 cells had strong antitumor activity in vivo with distinct mechanisms. However, immunological memory suitable for the generation of tumor-specific cytotoxic T lymphocytes was induced only when tumor-bearing mice received Th1 cell therapy, but not Th2 cell therapy. Thus it was strongly suggested that Th1-dominant immunity is critically important for the induction of antitumor cellular immunity in vivo. We also proposed that several immunomodulating protocols using interleukin (IL)-12, IL-12 gene, the natural killer T cell ligand α-galactosylceramide, or Th1 cytokine-conditioned dendritic cells might be useful strategies for the induction of Th1-dominant immunity essential for the development of tumor-specific immunotherapy.

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Introduction

The discovery of tumor-rejection antigen (TRA) peptides confirmed that cytotoxic T lymphocytes (CTLs) derived from tumor-bearing hosts can lyse autologous tumor cells in both human and mouse systems [25]. This finding has also encouraged us to develop tumor-specific immunotherapy using TRA peptide [6]. Although a variety of TRA peptides bound to major histocompatibility complex (MHC) class I molecules have been isolated from tumors, it remains difficult to achieve complete cure in tumor-bearing hosts by immunization with TRA peptide alone. This failure might be because of the lack of helper T cell activation by MHC class I-binding TRA peptide. It has been reported that the activation of the helper arm is essential to overcome immunosuppression in tumor-bearing mice and for the induction of tumor-specific CTLs [4, 16]. Therefore the development of a protocol to induce helper T cell activation at local tumor sites (local help) may be required to develop more efficient tumor-specific immunotherapy [8].

Recently, it has been demonstrated that the immune balance (Th1/Th2 balance) controlled by cytokines produced by Th1 and Th2 cells plays an important role in immunoregulation, including antitumor immunity [10]. Judging from the fact that interleukin (IL)-12, a Th1-activating cytokine, shows strong antitumor activity in vivo, Th1-dominant immunity is superior to Th2 immunity in the induction of antitumor immunity [24]. However, gene therapy using Th2 cytokines such as IL-4, IL-6, and IL-10 has also been reported to be effective for tumor immunotherapy [1]. Therefore it remains unclear how Th1 or Th2 immunity is involved in antitumor immunity in vivo.

In this paper, we first document the distinct role of antigen-specific Th1 or Th2 cells in tumor destruction

in vivo and then propose several methods for manipulation of Th1-dominant immunity, which may be essential for induction of cell-mediated antitumor immunity.

Materials and methods

Animals

Female C57BL/6 mice and BALB/c mice were purchased from Charles River Japan (Yokohama, Japan). Vα14 natural killer T (NKT) cell-deficient (Jα281$^{-/-}$) mice were kindly donated by Dr. M. Taniguchi, Graduate School of Medicine, Chiba University, Chiba, Japan. The mice were maintained in specific pathogen-free conditions and used for the experiments at 5–8 weeks of age.

Induction of Th1 and Th2 cells

Th1 and Th2 cells were induced from CD4$^+$CD45RB$^+$ naive Th cells isolated from OVA-specific DO11.10 TCR transgenic mice as described previously [14].

Adoptive tumor immunotherapy using Th1 and Th2 cells

A20-OVA, which were A20 B lymphoma cells transfected with the OVA gene, were inoculated intradermally into BALB/c mice. When the A20-OVA tumor mass become palpable, OVA-reactive Th1 or Th2 cells (2×10^7) were adoptively transferred into tumor-bearing mice. The detailed protocol for this therapeutic model was described in a previous report [12].

IL-12 cytokine gene therapy

IL-12 was kindly donated by the Genetics Institute, Cambridge, MA, USA. When the tumor mass become palpable (6–8 mm in diameter), IL-12 2000 U/mouse ip daily was injected into the mice for 4 days. Murine IL-12 genes, and p35 and p40 subunit genes, were kindly donated by the Genetics Institute. The p40 cDNA was subcloned into BCMGSNeo plasmid (generous gift of Dr. H. Karasuyama, Department of Immunology, Tokyo Metropolitan Institute, Tokyo, Japan) and the p35 cDNA was subcloned into CEP4 plasmid (Invitrogen, San Diego, CA, USA). BALB/c-derived A20 B lymphoma cells were first transfected with BCMGSNeo-p40, and then the cells were further transfected with CEP4-p35 plasmid by electroporation. A20 cells cotransfected with p40 and p35 genes were selected by culture with medium containing G418 0.5 mg/mL and hygromycin B 0.5 mg/mL. We then investigated the effect of IL-12 gene therapy on tumor growth in this IL-12 gene transfectant (IL-12-A20C1).

Detection of cytokine activity

Serum IL-4 or interferon (IFN)-γ activity was determined using the OptEIA™ Mouse IL-4 or OptEIA™ Mouse IFN-γ Set (PharMingen, San Diego, CA, USA). Total IL-12 activity was determined using the Intertest-12™ (Genzyme Corporation, Cambridge, MA, USA).

Determination of cytotoxicity

Cytotoxic activity was measured by 4-h ^{51}Cr-release assay. The percentage of specific chromium release was calculated as described previously [13].

Prevention of lung metastasis of B16-BL6-HM melanoma cells by combination α-galactosylceramide and IL-12

The α-galactosylceramide (α-GalCer) used for this study was donated by Kirin Brewery Co., Ltd., Tokyo, Japan. B16-BL6-HM cells 10^5 suspended in phosphate-buffered saline 0.1 mL were injected intravenously into C57BL/6 mice to establish pulmonary metastases. One day after tumor inoculation, the mice received an injection of α-GalCer 2 μg/mouse iv daily and/or IL-12 2000 U/mouse ip daily for 3 days. Fourteen days after tumor inoculation, lung metastases were counted as described previously [11].

Induction of dendritic cells from bone marrow

Bone marrow cells obtained from BALB/c mouse femur were cultured under three distinct culture conditions in 12-well plates (Costar, Corning, NY, USA) for 4 days. Bone marrow dendritic cells-zero (BMDC0) were induced by culture of bone marrow cells (5×10^6 cells/well) in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF) 20 ng/mL and IL-3 20 ng/mL. BMDC1 were induced by culture of bone marrow cells in the presence of IFN-γ 20 ng/mL and IL-12 20 U/mL in addition to GM-CSF + IL-3. BMDC2 were induced by culture of bone marrow cells in the presence of IL-4 20 ng/mL in addition to GM-CSF + IL-3. The detailed culture conditions and assay method for evaluating the supporting effect on Th differentiation and CTL generation were described previously [20, 21].

Results

Role of antigen-specific Th1 and Th2 cells in tumor eradication in vivo

OVA-specific Th1 and Th2 cells were induced from naive Th cells obtained from OVA-TCR transgenic mice. As summarized in Table 1, Th1 and Th2 cells demonstrated different biological functions and pattern of cytokine production. Th1 exhibited greater cytotoxicity than Th2 cells in perforin-, tumor necrosis factor (TNF)-α-, and Fas/FasL-mediated cytotoxicity. Moreover, Th1 cells revealed a strong adhesion mechanism through leukocyte function-associated antigen (LFA)-1/intracellular adhesion molecule (ICAM)-1 molecules and produced higher cytokine levels compared with Th2 cells.

Table 1 Functional differences between Th1 and Th2 cells (*IL-4* interleukin 4, *IFN-γ* gamma interferon, *TNF-α* tumor necrosis factor alpha, *LFA* leukocyte function-associated antigen, *ICAM-1* intracellular adhesion molecule 1, *AICD* activation-induced cell death, *RANTES* regulated upon activation, normal T cell expressed and secreted, *MIP* macrophage inflammatory protein)

Characteristic	Th1 cells	Th2 cells
Cytokines		
IL-4	–	+ + +
IFN-γ	+ + +	–
Cytotoxic activity		
Perforin	+ + +	±
TNF-α	+ + +	±
Fas/FasL	+ + +	±
Adhesion		
LFA-1/ICAM-1	+ + +	–
Apoptosis		
AICD	+ + +	+
Chemokines		
RANTES, MIP-1α, -β	+ + +	+

To clarify the precise role of antigen-specific Th1 and Th2 cells in tumor eradication, OVA-specific Th1 or Th2 cells 2×10^7 were adoptively transferred into BALB/c mice bearing A20-OVA tumor masses. As shown in Fig. 1A, both Th1 and Th2 cells exhibited strong antitumor activity in vivo and all mice which received Th1 and Th2 cells experienced complete tumor regression. Interestingly, the in vivo antitumor activity of Th1 and Th2 cells occurred through different mechanisms: Th1 cells eradicated tumor through cell-mediated immunity, but Th2 cells eradicated the tumor mass by necrosis (Fig. 1C–H). This therapeutic effect of Th1 and Th2 cells was abrogated in $RAG2^{-/-}$ mice and reconstituted by the transfer of $CD8^+$ T cells (data not shown). Therefore the therapeutic effect of Th1 and Th2 cells appeared to be achieved by cooperation with $CD8^+$ T cells in the tumor-bearing host. However, tumor-specific CTLs were generated only from spleen cells of mice cured of tumor by Th1 cell therapy, but not Th2 cell therapy (Fig. 1B). It was also demonstrated that IFN-γ-producing cytotoxic T lymphocyte-type 1 (Tc1) were induced in mice cured by Th1 cell therapy, while IL-4-producing Tc2 were induced in mice cured by Th2-cell therapy (data not shown). Therefore Th1 cell therapy appears to induce antitumor cell-mediated immunity in vivo.

As shown in Fig. 2, we also found that such tumor-specific Th1 cells could be induced from tumor-infiltrating lymphocytes (TILs) by culture with autologous tumor cells in the presence of IL-2 + IL-12. These data strongly suggest that Th1 cells would be applicapable to adoptive tumor immunotherapy in both animal and human systems.

Use of IL-12 or IL-12 gene to induce Th1-dominant immunity

The simplest method to induce Th1-dominant immunity in tumor-bearing hosts is the use of IL-12. As shown in our previous report [15], in vivo administration of IL-12 caused the elevation of serum IFN-γ levels and activation of killer cell generation in vivo. In parallel with such immunopotentiating effects, IL-12 exhibited potent antitumor activity in vivo against subcutaneously injected B16-BL-6 melanoma mass (Fig. 3A). Interestingly, a strong antitumor activity was also demonstrated even in NKT-deficient mice (Fig. 3A and B). Therefore NKT cells are not always necessary for IL-12-induced tumor immunotherapy. Using B lymphoma cells (A20C1) transfected with IL-12 p35, p40 genes, it was demonstrated that A20C1 showed a strong tumor vaccine effect, since the growth of A20 tumor mass was prevented by vaccination with A20C1 cells. However, the growth of BAMC-1 cells was not affected by vaccination with A20C1 cells. Thus IL-12 transfectants are able to induce systemic tumor-specific immunity through the activation of Th1-dominant immunity.

In the case of B16-BL6 melanoma cells, which express low levels of MHC class I molecules, it is very

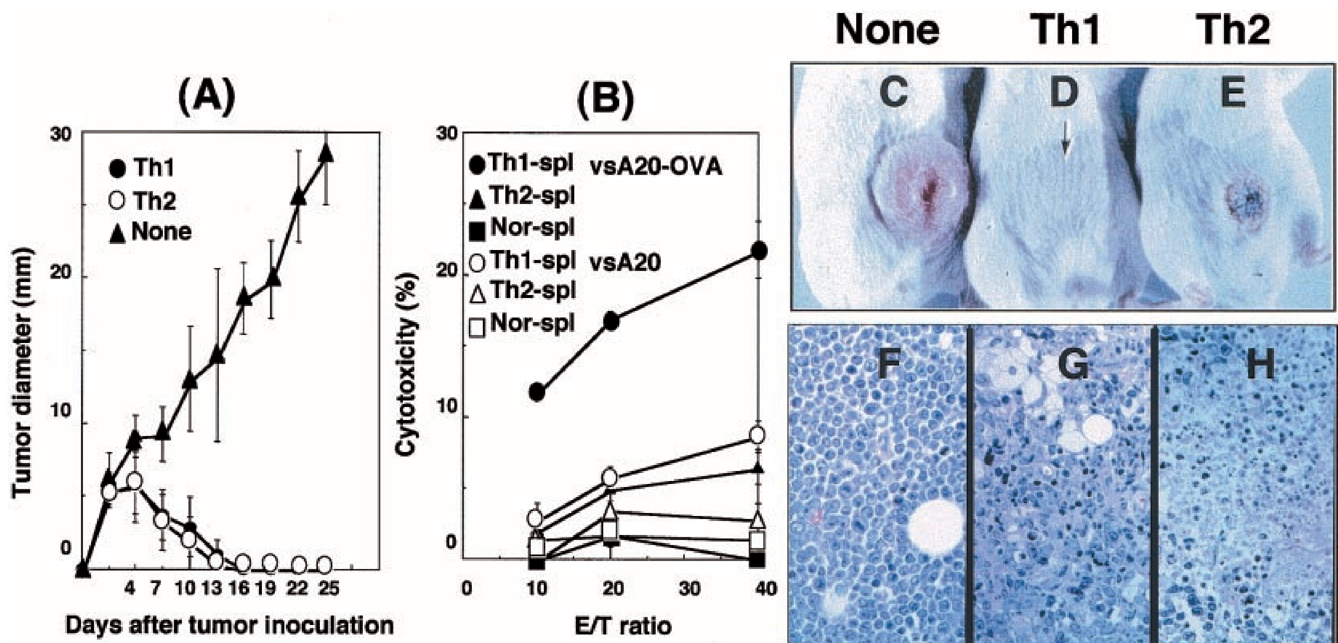


Fig. 1 The separate roles of Th1 and Th2 cells in the eradication of established tumors in vivo. **A** OVA-specific Th1 cells (closed circles), OVA-specific Th2 cells (open circles), or saline (closed triangles) were injected intravenously into BALB/c mice bearing 6–8 mm A20-OVA tumor masses. Two perpendicular diameters of the tumor mass were measured. **B** Mice with complete regression of A20-OVA tumors after Th1 (Th1-spl) or Th2 cell therapy (Th2-spl) were killed, and their spleen cells were restimulated with mitomycin C-treated A20-OVA by mixed lymphocyte tumor reaction. After four-day culture, cells were harvested and their cytotoxicity against A20-OVA (closed symbols) or A20 (open symbols) was determined. **C–E** Typical tumor growth or regression pattern in mice administered saline (**C**), Th1 (**D**), or Th2 (**E**). **F–H** Histochemical analysis of the cellular mechanisms underlying Th1- (**G**) or Th2-mediated tumor eradication (**H**). **F** Mice administered saline

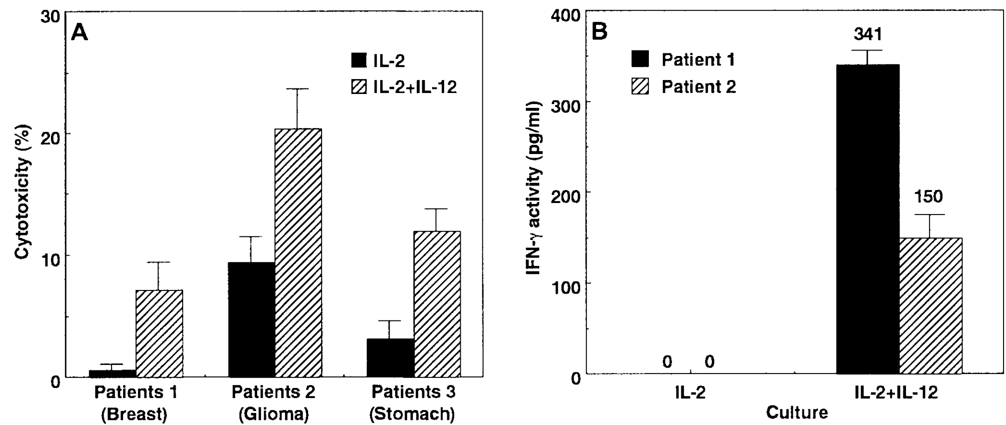


Fig. 2 Generation of tumor-reactive Th1 cells from human TILs. TILs obtained from a primary solid tumor were cultured with IL-2 10 μ/mL or IL-2 + IL-12 10 U/mL. After expansion of TILs, $CD4^+$ TILs were isolated by fluorescence-assisted cell sorting and their cytotoxicity against autologous tumor cells was determined by 4-h ^{51}Cr-release assay (**A**). **B** IFN-γ levels produced by $CD4^+$ TILs cultured with autologous tumor cells for 2 days were measured by ELISA

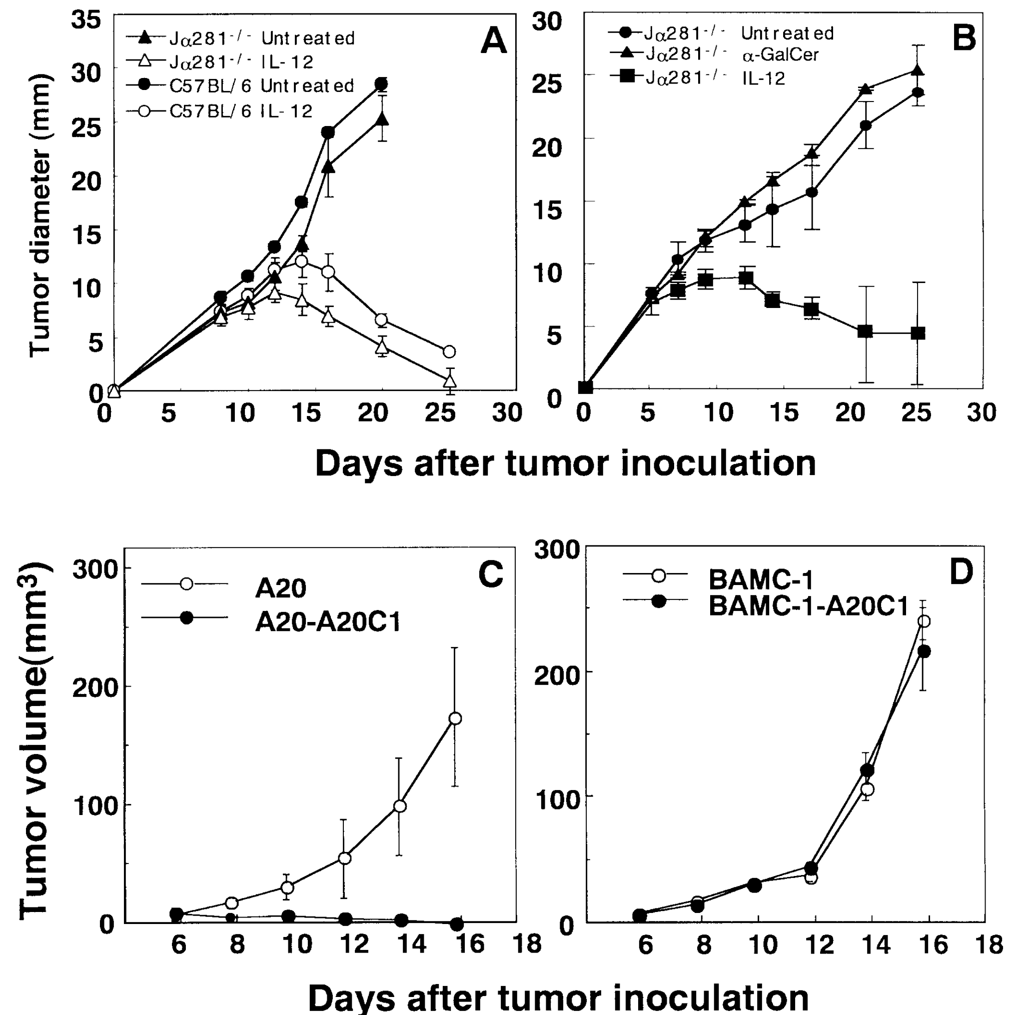


Fig. 3 IL-12 in antitumor cytokine and gene therapy. **A** MBL-2 T lymphoma cells were inoculated intradermally into wild-type C57BL/6 mice (circles) or Ja281$^{-/-}$ NKT-deficient mice (triangles). When tumor mass became palpable, the tumor-bearing mice were injected intraperitoneally with saline (closed symbols) or IL-12 2000 U/mouse (open symbols) for 4 days. **B** B16-BL-6HM melanoma cells were inoculated into NKT-deficient mice. Then the tumor-bearing mice were given saline (closed circles), α-GalCer (closed triangles), or IL-12 (closed squares). **C**, **D** BALB/c mice were intradermally inoculated with viable A20 (**C**) or BAMC-1 (**D**) on the right side of the abdomen. When tumor mass became palpable (2–3 mm), mitomycin C-treated IL-12-A20C1 cells were inoculated into the opposite side of abdomen as tumor vaccine (closed circles). As a control, saline was injected into tumor-bearing mice (open symbols)

difficult to induce CTL and IFN-γ production in the mixed lymphocyte tumor reaction. However, introduction of IL-12 genes into the tumor caused a marked increase in IFN-γ production. Moreover, both CTL generation and IFN-γ production were enhanced when B16-BL-6 melanoma cells were transfected with IL-12 gene + B7.1 gene (Fig. 4). Thus IL-12 and IL-12 genes coupled with costimulatory genes may provide a useful tool for the induction of Th1-dominant immunity.

Use of α-GalCer to activate Th1-dominant immunity

In vivo administration of the NKT cell ligand α-GalCer resulted in the activation of NKT cells through the

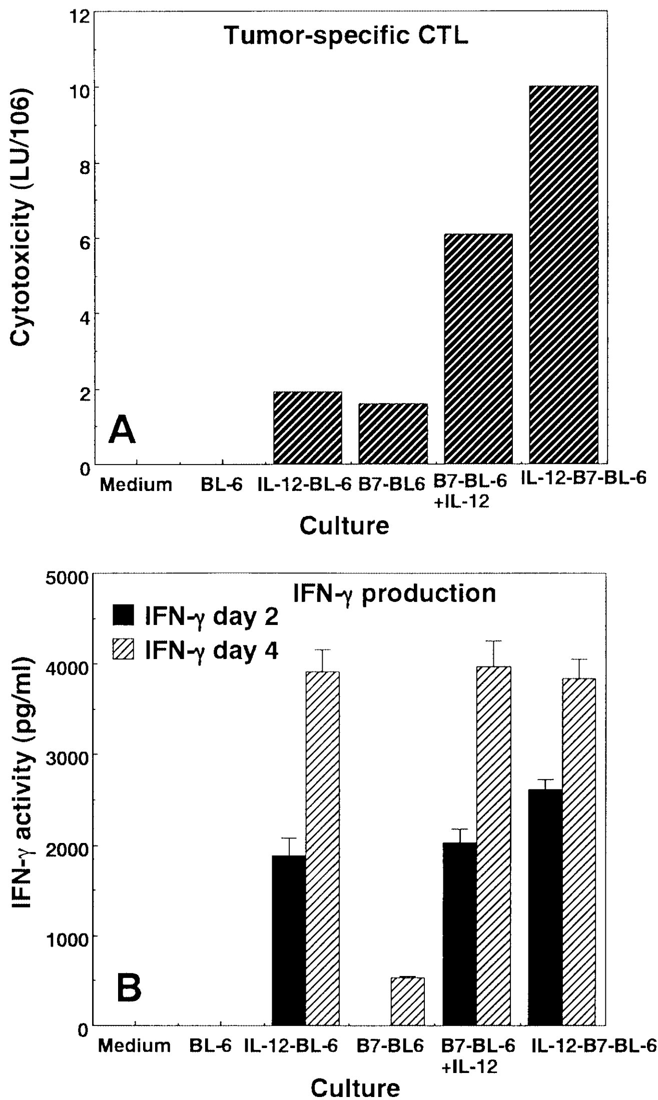


Fig. 4 Efficient induction of tumor-specific CTLs and IFN-γ production in mixed lymphocyte tumor reaction by stimulation with tumor cells transfected with B7.1 and IL-12 gene. C57BL/6 mouse spleen cells were cocultured with RPMI medium containing 10% fetal calf serum, B16-BL6 melanoma cells (Bl-6), B7.1 gene-transfected B16-BL6 (B7-BL6), IL-12 gene-transfected B16-BL-6 (IL-12-BL-6), or B16-Bl6 transfected with both B7.1 and IL-12 gene (IL-12-B7-BL-6) for 4 days. After culture, cells were harvested and their cytotoxicity against B16-BL-6 was determined (**A**). **B** IFN-γ levels in culture supernatants of MLTR were determined by ELISA at 2 and 4 days of culture

production of IL-12 by dendritic cells and upregulation of IL-12 receptor (IL-12R) on NKT cells [7]. As shown in Fig. 5, α-GalCer administration induced the production of IL-12, IFN-γ, and IL-4 and the generation of natural killer activity. This immunopotentiating effect of α-GalCer was not observed in NKT-deficient mice or CD1d$^{-/-}$ mice, indicating that α-GalCer activates NKT cells in a CD1d^{+}DC-dependent manner (data not shown). It was also demonstrated that α-GalCer acts synergistically with IL-12 to enhance IFN-γ production and natural killer activity, although IL-4 production was inhibited (Fig. 6). Moreover, the antimetastatic activity of α-GalCer was enhanced by coadministration with IL-12 (Fig. 7A). The therapeutic effect of α-GalCer is abolished in NKT-deficient mice, while that of IL-12 is unchanged. Therefore α-GalCer and IL-12 may have a synergistic effect through separate immunopotentiating mechanisms.

These results indicate that combined administration of α-GalCer and IL-12 appeared to induce Th1-dominant immunity in tumor-bearing mice. However, α-GalCer did not show any significant antitumor activity against intradermally implanted solid tumor masses (data not shown). Thus NKT cells appeared to be effective against metastatic but not against solid tumors.

As shown in Fig. 8, administration of α-GalCer resulted in activation of CD4^{+} T cells and CD8^{+} T and B cells in addition to NKT cells. The α-GalCer-activated CD69^{+}CD8^{+} cells exhibited strong cytotoxicity and IFN-γ-producing ability, indicating that the acquisition of CD69 antigen is related to functional differentiation of CD8^{+} T cells. α-GalCer-induced activation of acquired immunity was induced in neither NKT-deficient mice nor CD1$^{-/-}$ mice (data not shown). Therefore NKT/dendritic cell interaction appears to be involved in the bridging between innate and acquired immunity.

Induction of Th1-dominant immunity by dendritic cell-based vaccination

Finally, we investigated which types of dendritic cell are suitable for the induction of Th1-dominant immunity. Three distinct dendritic cell subsets were induced from bone marrow cells by culture with GM-CSF + IL-3 (neutral conditions), GM-CSF + IL-3 + IL-12 + IFN-γ (Th1 conditions), or GM-CSF + IL-3 + IL-4 (Th2 conditions) for 4 days. BMDCs induced under neutral, Th1-positive, and Th2-positive conditions were designated BMDC0, BMDC1, and BMDC2, respectively. As shown in Fig. 9A, BMDC1 induced from bone marrow by Th1-cytokines expressed the highest levels of MHC class I, class II, and costimulatory molecules compared with BMDC0 or BMDC2. BMDC1 produced high levels of IL-12, while BMDC2 produced lower levels of IL-12 (Fig. 9B). Moreover, BMDC1 supported the differentiation of IFN-γ-producing Th1 cells from naive Th cells obtained from DO11.10 TCR transgenic mice. In contrast, BMDC2 supported the development of IL-4-producing Th2 cells (Fig. 9C). We also investigated the functional heretogeneity of BMDC subsets in the generation of allogeneic CTLs by mixed lymphocyte culture. As shown in Fig. 9D, BMDC1 stimulated CTL generation, while BMDC2 did not induce CTL. BMDC0 stimulation was intermediate between the two. Thus BMDC1 conditioned by Th1-biasing cytokines appear to induce Th1-dominant immunity. Once dendritic cells were conditioned by Th1-cytokines, they could replace helper T cell function and support the generation of CTLs from naive CD8^{+} T cells in the absence of helper T cells (data not shown).

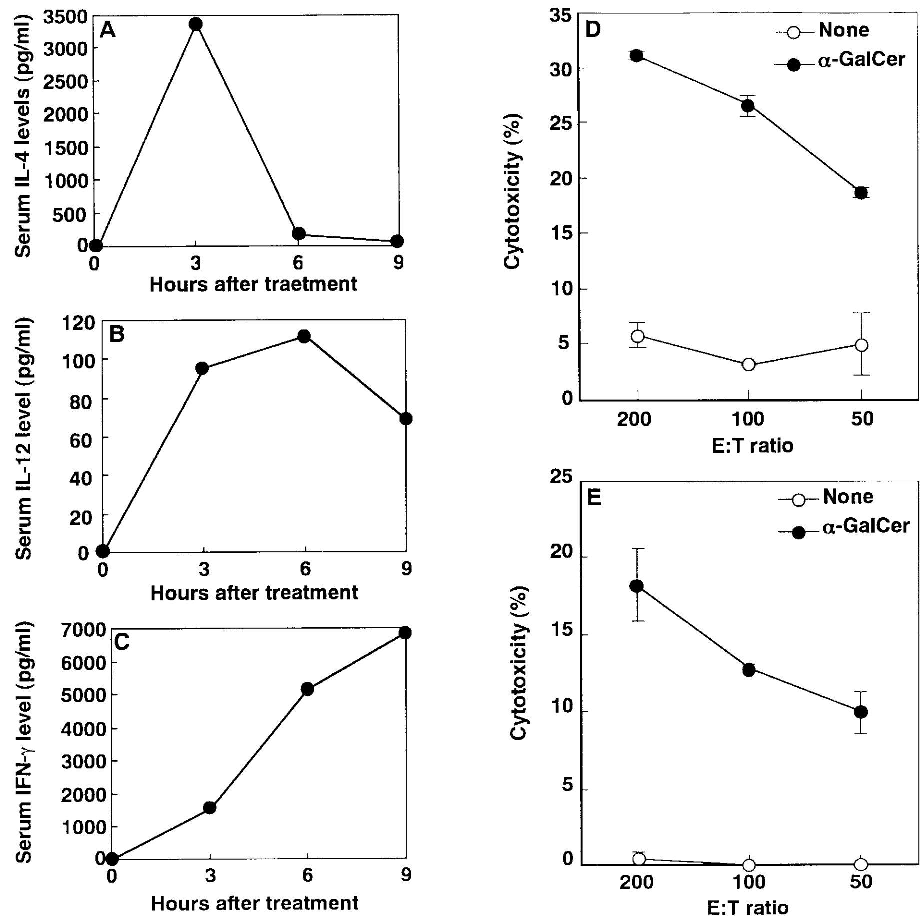


Fig. 5 Induction of cytokine production and natural killer activity by α-GalCer administration in vivo. **A–C** serum IL-4, IL-12, and IFN-γ levels were determined by ELISA at various times after α-GalCer administration. **D**, **E** Spleen cells were prepared from C57BL/6 mice administered α-GalCer 4 h before the experiment. Natural killer activity against YAC-1 cells (D) or B16-Bl-6HM (E) was measured by 4-h ^{51}Cr-release assay

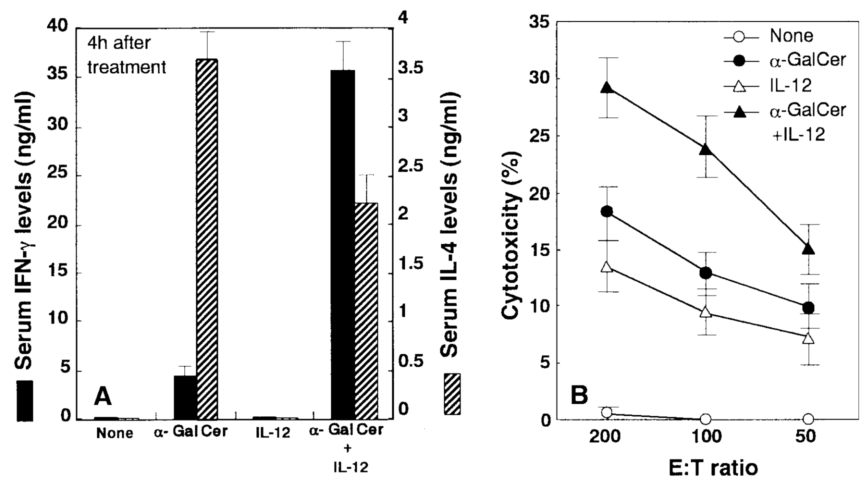


Fig. 6 Synergistic effect of α-GalCer and IL-12 on the elevation of serum IFN-γ levels and natural killer activity. C57BL/6 mice were intravenously injected with α-GalCer and/or intraperitoneally injected with IL-12. **A** Four hours after treatment the mice were killed and serum IFN-γ (solid bars) and IL-4 levels (hatched bars) measured by ELISA. **B** Natural killer activity against B16-BL-6HM in spleen cells was determined by 4-h ^{51}Cr-release assay

Discussion

The final goal of tumor immunotherapy is the development of an efficient method to overcome immunosuppression in tumor-bearing hosts and to induce tumor-specific immunity in vivo. Many investigators have used TRA peptides to induce tumor-specific CTLs in vivo [5, 6]. However, it appears difficult to induce CTLs without the activation of helper T cells.

Recently, it has been accepted that the Th1/Th2 balance regulated by two distinct Th subsets is critical in the onset of various immune diseases [10, 14]. It has been believed that IL-12-induced Th1-dominant immunity is preferable for the induction of antitumor immunity [17, 24]. However, we have found that both Th1 and Th2 cells exhibit potent antitumor activity in vivo through separate mechanisms [12]. Th1 cells eradicate tumor mass by conferring cellular immunity, while Th2 cells reject tumors by necrosis mechanisms

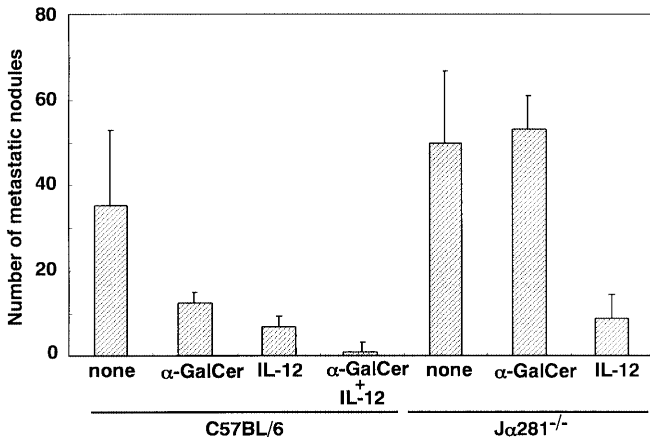
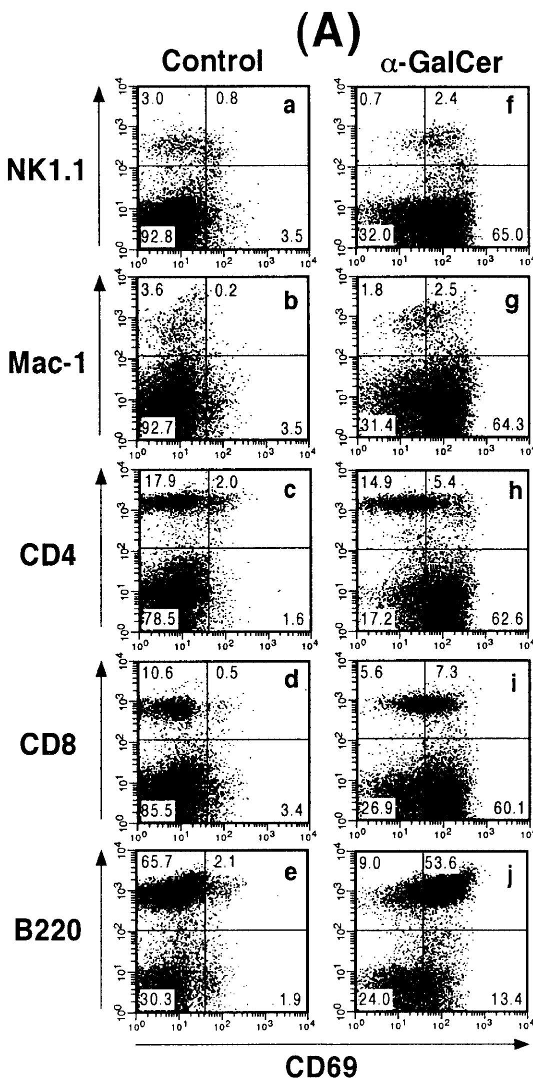


Fig. 7 Synergistic therapeutic effect of α-GalCer and IL-12 on the prevention of tumor metastasis to the lung. Wild-type C57BL/6 mice or Jα281$^{-/-}$ NKT-deficient mice were intravenously inoculated with B16-BL-6HM to establish lung metastases. One day after tumor inoculation, the mice received nothing, or were administered α-GalCer, IL-12, or α-GalCer + IL-12 for 3 days. Lung tumor nodules were counted 14 days after tumor inoculation

mediated by inflammatory cell infiltration. Moreover, it was demonstrated that the mice cured of tumor by Th1 cell therapy acquire an immunological memory allowing CTL generation, but that this does not occur in mice treated with Th2 cell therapy (Fig. 1). Our results are consistent with the finding that mice cured of tumor with IL-4-gene therapy reject tumors through inflammatory mechanisms and do not acquire immunological memory [23]. We also demonstrated that IFN-γ-producing CTLs are induced in the mice cured by Th1 cell therapy but not from those which receive Th2 cell therapy. These results suggest that the Th1/Tc1 circuit is important for the induction of cellular immunity in tumor-bearing mice. Taken together, these findings and the fact that Th2 cells produce high levels of IL-6 and IL-10, which cause cachexia in tumor-bearing hosts [22], indicate that Th1-dominant immunity is superior to Th2 immunity for application in tumor immunotherapy.

As tools for the induction of Th1-dominant immunity, we propose four different protocols: 1) adoptive



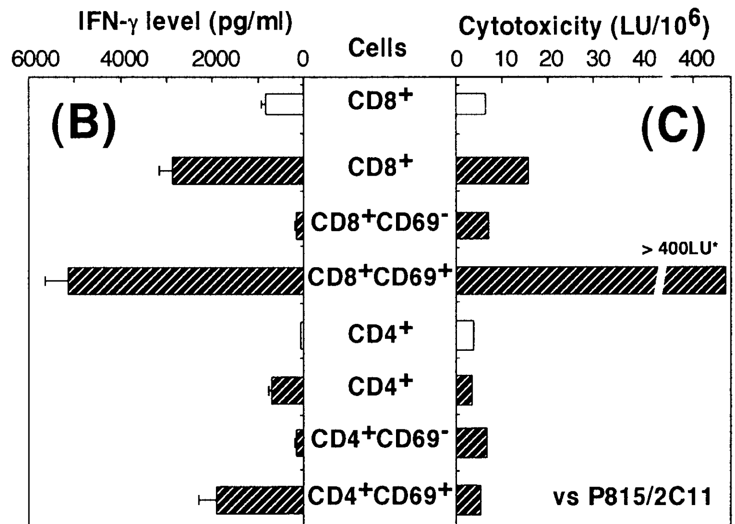


Fig. 8 Bridging between innate and acquired immunity by NKT cells. **A** C57BL/6 mice were injected with α-GalCer. Then CD69 expression on NK1.1^{+} cells (a, f), Mϕ (b, g), CD4^{+} T cells (c, h), CD8^{+} T cells (d, i), and B cells (e, j) obtained from untreated control mice (a–e) or α-GalCer-treated mice (f–j) was measured by flowcytometry. **B** CD69^{+}CD8^{+} T cells and CD69^{-}CD8^{+} T cells were isolated from α-GalCer-treated C57BL/6 mouse spleen cells by FACSVantage^{TM} (Becton Dickinson, San Jose, CA, USA) and their IFN-γ-producing ability was measured by ELISA. **C** Cytotoxicity against P815 cells was determined by reverse antibody-dependent cellular cytotoxicity assay in the presence of anti-CD3 monoclonal antibody 1 μg/mL

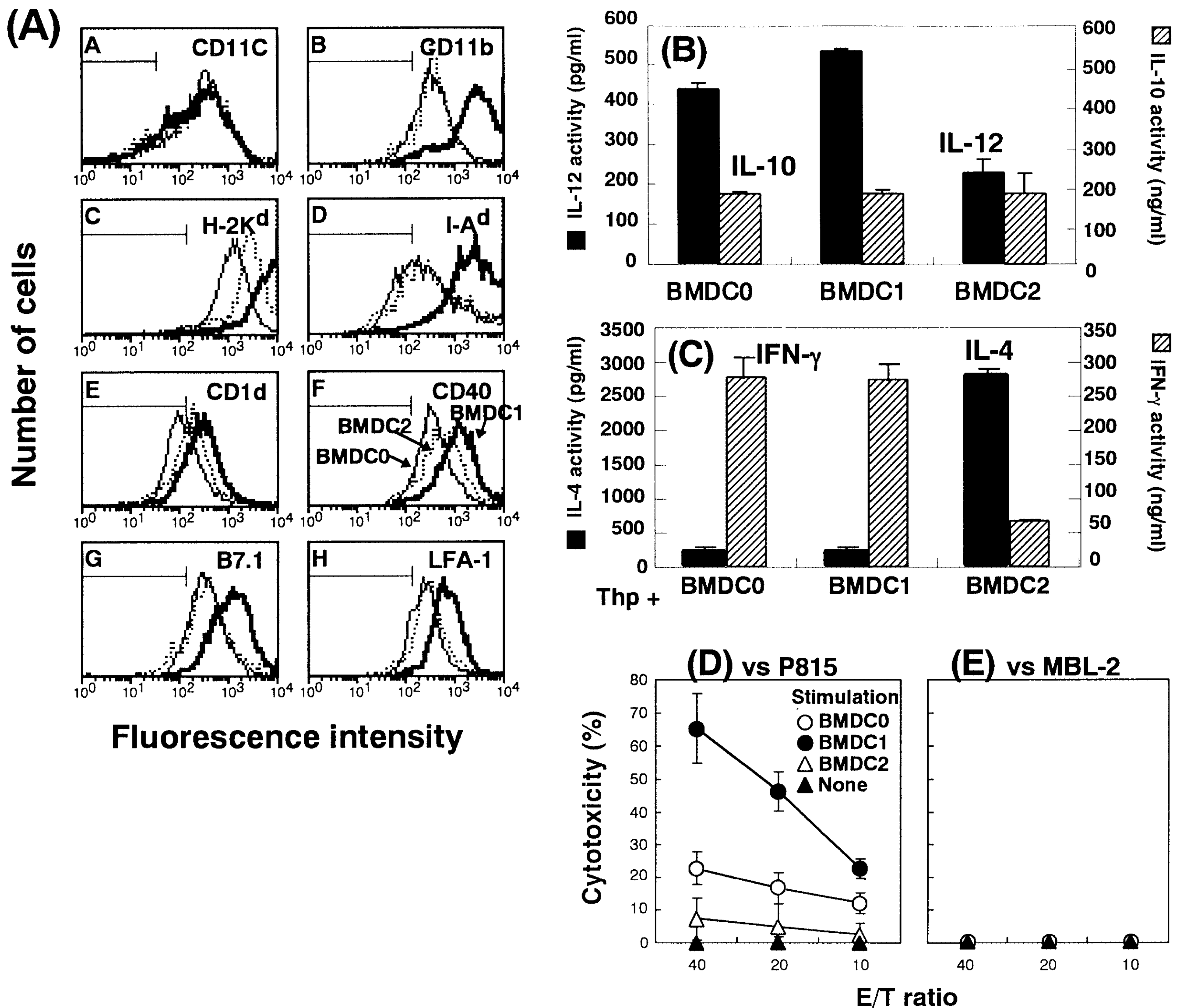


Fig. 9 Functional heterogeneity of BMDC subsets. BMDC0, BMDC1, and BMDC2 cells were induced from BALB/c mouse bone marrow cells as described in the Materials and methods section. **A** Phenotypic characterization of BMDC subsets was carried out using FACSCalibure™ (Becton Dickinson). BMDC0 (solid line), BMDC1 (bold line), and BMDC2 (dotted line). **B** The levels of IL-12 (solid bars) and IL-10 (hatched bars) in culture supernatants of lipopolysaccharide-stimulated BMDC subsets were determined by ELISA. **C** Naive Th cells (Thp) obtained from DO11.10 transgenic mice were cocultured with BMDC subsets for 7 days. Then the cells were restimulated with OVA peptide in the presence of BMDC for 24 h. The IFN-γ and IL-4 levels in culture supernatants were measured by ELISA. **D**, **E** C57BL/6 mouse spleen cells were cultured alone (solid triangles), or with BMDC0 (open circles), BMDC1 (closed circles), or BMDC2 (open triangles) for 4 days. Then the cells were harvested and their cytotoxicity against antigenic tumor P815 (**D**) or unrelated tumor MBL-2 (**E**) cells was determined by 4-h ^{51}Cr-release assay

immunotherapy by antigen-specific Th1 cells; 2) application of IL-12 or IL-12 genes; 3) induction of Th1-dominant immunity using immunopotentiators such as the NKT cell ligand α-GalCer; and 4) application of Th1-cytokine-conditioned dendritic cells, such as BMDC1 or DC1.

As shown in Figs. 3 and 4, IL-12 and IL-2 genes are useful to induce tumor rejection through the activation of Th1-dominant immunity. However, it has been reported that IL-12 administration can result in unexpected side effects [19]. Therefore future studies must clarify which gene(s) is critical in the control of susceptibility to IL-12 administration. To decrease the side effects of IL-12, we developed combination therapy using the NKT cell ligand α-GalCer + IL-12. As previously demonstrated by us [7], α-GalCer can activate only $CD4^+NK1.1^+$NKT cells, but not mainstream $CD4^+$ T cells, $CD8^+$ T cells or $CD4^-NK1.1^+$ NK cells. α-GalCer-activated NKT cells produce high levels of cytokines and exhibit strong natural killer activity, and these NKT cell functions were enhanced by combined administration with IL-12 (Figs. 6 and 7). Therefore α-GalCer acts synergistically with IL-12 to prevent tumor cell metastasis. However, α-GalCer

does not reject intradermally injected tumor masses. A unique biological function of α-GalCer is activation of acquired immunity in addition to NKT cells. As shown in Fig. 8, in vivo administration of α-GalCer resulted in the induction of CD69 early-activation marker on CD4, CD8, Mϕ, and B cells in addition to NKT cells. This phenomenon is not a trivial marker change, but is related to functional differentiation since α-GalCer-activated $CD69^+CD8^+$ T cells, but not $CD69^-CD8^+$ T cells, showed significant cytotoxicity and IFN-γ-producing ability. Thus NKT cells play an important role in bridging between innate and acquired immunity, which may facilitate the induction of Th1 and CTLs.

In addition to NKT cells, dendritic cells, one of the most "professional" types of antigen-presenting cells, also play an important role in bridging between innate and acquired immunity [2]. Recently, it has been demonstrated that there are functional heterogeneities in dendritic cell subsets. In mice, $CD8\alpha^+$ lymphoid dendritic cells produce IFN-γ and support the development of Th1 cells, while myeloid dendritic cells support the differentiation of Th2 cells [3, 9, 18]. In this paper, we demonstrate that $CD11c^+CD11b^+$ myeloid dendritic cells have functional heterogeneities in the development of Th1, Th2, and CTLs [20, 21]. BMDC1 conditioned by Th1-biasing cytokines functioned the most efficiently to support Th1-dominant immunity suitable for CTL generation. Recently, dendritic cells conditioned by GM-CSF + IL-4 have been used in a clinical trial of dendritic cell-based tumor vaccination therapy [26]. However, based on other recent findings [3, 9, 18, 20, 21], it is necessary to confirm the functions of dendritic cells before their application in clinical trials.

Our results suggest that Th1-dominant immunity may be a rational strategy for the induction of antitumor immunity. Although we propose several methods to manipulate Th1-dominant immunity, it is necessary to evaluate TRA peptides bound on MHC class II molecules [27], which will make it easier to induce tumor-specific Th1 cells.

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