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ICOS costimulates invariant NKT cell activation☆

Hiroshi Kaneda[a,b,1], Kazuyoshi Takeda[a,*,1], Tsuyoshi Ota[a,b], Yuki Kaduka[a,b], Hisaya Akiba[a], Yoshinori Ikarashi[c], Hiro Wakasugi[c], Mitchell Kronenberg[d], Katsuyuki Kinoshita[b], Hideo Yagita[a], Ko Okumura[a]

[a] *Department of Immunology, Juntendo University School of Medicine, Tokyo 113-8421, Japan*
[b] *Department of Obstetric and Genecology, Juntendo University School of Medicine, Tokyo 113-8421, Japan*
[c] *Pharmacology Division, National Cancer Center Research Institute, Tokyo 104-0045, Japan*
[d] *Division of Developmental Immunology, La Jolla Institute for Allergy and Immunology, San Diego, CA 92121, USA*

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Abstract

It has been reported that costimulatory molecules, CD80/86–CD28 and CD154–CD40, critically contribute to activation of CD1d-restricted invariant NKT (iNKT) cells. Here we have demonstrated that ICOS, a new member of the CD28 family, plays a substantial role in iNKT cell activation. iNKT cells constitutively expressed ICOS as well as CD28 independently, and ICOS expression was further up-regulated 2–3 days after α-galactosylceramide (α-GalCer) treatment. Blockade of ICOS-mediated costimulation by administration of anti-ICOS ligand (B7RP-1) mAb or by ICOS gene knockout substantially inhibited α-GalCer-induced IFN-γ and IL-4 production, cytotoxic activity, and anti-metastatic effect. Moreover, blockade of both B7RP-1–ICOS and CD80/86–CD28 interactions mostly abolished the α-GalCer-induced immune responses. These findings indicate that iNKT cell activation is regulated by CD28 and IOCS independently.

Keywords: NKT cells; α-Galactosylceramide; ICOS; CD28; IFN-γ; IL-4; Cytotoxicity; Anti-tumor effect

The typical invariant NKT (iNKT) cells coexpress a semi-invariant T cell receptor (TCR), consisting of an invariant TCR Vα14Jα18 chain (Vα24Jα15 in humans) and a TCR β-chain that is largely biased towards Vβ8.2 (Vβ11 in humans), Vβ2, and Vβ7, as well as NK cell receptors such as NK1.1 [1,2]. Although the physiological antigens (Ags) for iNKT cells still remain unclear, this semi-invariant TCR recognizes glycolipid antigens, such as α-galactosylceramide (α-GalCer) and its analogs, presented on an MHC class I-like molecule, CD1d [1,2]. iNKT cells secrete various cytokines, including both IFN-γ and IL-4, promptly after TCR ligation [1,2]. Accordingly, iNKT cells are thought to be potent immune-regulatory cells and their activation by ligands has been shown to be a powerful means to modulate various immune responses [1,2].

In addition to the regulation by specific ligands through TCR, activation of iNKT cells is critically regulated by costimulatory signals provided by antigen-presenting cells (APCs). We and others have previously demonstrated that a costimulatory signal mediated through the CD80/86–CD28 interaction is required for optimal activation of iNKT cell functions in response to α-GalCer in vitro and in vivo [3,4]. Moreover, we have also demonstrated that the CD154–CD40 interaction is critically involved in IFN-γ production and induction of Th1-type responses by α-GalCer-activated

☆ *Abbreviations:* iNKT, invariant NKT; TCR, T cell receptor; α-GalCer, α-galactosylceramide; APCs, antigen-presenting cells; B7RP-1, B7-related protein-1.

* Corresponding author. Fax: +81 3 3813 0421.
E-mail address: ktakeda@med.juntendo.ac.jp (K. Takeda).

[1] These authors contributed equally to this work.

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iNKT cells [3,5]. Recently, ICOS, a new member of the CD28 family of costimulatory receptors, was identified to be inducibly expressed on conventional T cell upon activation [6]. It has been reported that the interaction of ICOS with its ligand, B7-related protein-1 (B7RP-1), on APCs is important for activation, proliferation, and cytokine production of Ag-primed conventional T cells, and preferentially leads to the generation of Th2 cells and controls Th1 responses [6–11]. Here, we demonstrated a critical contribution of ICOS to α-GalCer-induced iNKT cell activation and functions.

Materials and methods

Mice. Wild-type (WT) C57BL/6 (B6) mice were obtained from Charles River Japan (Yokohama, Japan). CD28-deficient ($CD28^{-/-}$) or ICOS-deficient ($ICOS^{-/-}$) B6 mice were kindly provided by Dr. Ryo Abe, Research Institute of Biological Science, Science University of Tokyo [12]. All mice were maintained under specific pathogen-free conditions and used in accordance with the institutional guidelines of Juntendo University.

Reagents. A synthetic form of α-GalCer was obtained from Kirin Brewery (Gunma, Japan). PE-conjugated tetrameric CD1d molecules loaded with α-GalCer (α-GalCer/CD1d) were prepared as described [13]. Anti-mouse CD80 mAb (RM80, rat IgG2a), anti-CD86 mAb (PO.3, rat IgG2b), and anti-B7RP-1 mAb (HK5.3, rat IgG2a) were prepared as described previously [14,15]. Control rat IgG was purchased from Sigma (St. Louis, MO).

Flow cytometric analysis. Mononuclear cells (MNC) were prepared from spleen and liver as described [3]. After pre-incubation with anti-mouse CD16/32 (2.4G2) mAb to avoid non-specific binding of mAbs to FcγR, surface molecules expressed on iNKT cells were analyzed on electronically gated α-GalCer/CD1d tetramer^{+} cells by four-color flow cytometry using a FACSCaliber (BD Bioscience, San Jose, CA). Surface molecules were stained with FITC-conjugated anti-NK1.1 mAb (PK136), PE-Cy5-conjugated anti-mouse ICOS mAb (7E.17G9), APC-conjugated anti-mouse CD28 mAb (37.51), or FITC-, PE-Cy5-, or APC-conjugated isotype-matched control mAbs. All these reagents were purchased from eBioscience (San Diego, CA).

Cell preparation and in vitro stimulation. Freshly isolated splenic MNC (5×10^5) from naïve or α-GalCer-primed mice were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated FCS, 2 mM L-glutamine, and 25 mM $NaHCO_3$ in humidified 5% CO_2 at 37 °C in 96-well U-bottomed plates (Coster, Cambridge, MA) as previously described [3]. Cells were stimulated with 100 ng/ml α-GalCer or vehicle (0.1% DMSO) in the presence (10 μg/ml) or absence of control rat Ig, anti-CD80 mAb, anti-CD86 mAb, and/or anti-B7RP-1 mAb. After 48 h, the cell-free culture supernatants were harvested to determine IFN-γ and IL-4 levels by ELISA.

Coculture of iNKT cells and DC. Freshly isolated hepatic MNC were stained with PE-conjugated α-GalCer/CD1d tetramer, and the positive cells were enriched by autoMACS using anti-PE microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's instructions. The enriched iNKT cells were then sorted on a FACS Vantage (BD Bioscience, San Jose, CA) to obtain highly purified (98–99%) iNKT cells. Splenic dendritic cells (DC) were prepared according to the reported method [16]. Purified iNKT cells (1×10^5) and DC (5×10^4) were cocultured as previously described [4,5] with 100 ng/ml α-GalCer or vehicle (0.1% DMSO) in the presence or absence of 10 μg/ml control rat Ig, anti-CD80 mAb, anti-CD86 mAb, and/or anti-B7RP-1 mAb. After 48 h, the cell-free culture supernatants were harvested to determine IFN-γ and IL-4 levels by ELISA.

In vivo treatment with α-GalCer and mAb. In most experiments, mice were i.p. injected with 2 μg α-GalCer in 200 μl PBS containing 0.1% DMSO. 0.1% DMSO in PBS was used as the vehicle control. Some mice were primed by i.p. injection of α-GalCer 3 days before. Some mice were i.p. administered with 300 μg control Ig, anti-CD80 mAb, anti-CD86 mAb, and/or anti-B7RP-1 mAb 1.5 days before the last α-GalCer injection. Sera were periodically obtained at 0–24 h after the last α-GalCer injection. IFN-γ and IL-4 levels in the sera were determined by ELISA. Hepatic and splenic MNC at 24 h were subjected to cytotoxic activity.

ELISA. IFN-γ and IL-4 levels in the culture supernatants or the sera were determined by using mouse IFN-γ or IL-4-specific ELISA kits (Ready-SET-Go!, eBioscience) according to the manufacturer's instructions.

Cytotoxicity assay. Cytotoxic activity of hepatic and splenic MNC was tested against NK-sensitive YAC-1 cells and NK-resistant P815 target cells by a standard 4-h ^{51}Cr release assay as previously described [3].

Experimental lung metastases. Log-phase cultures of B16 melanoma cells were harvested with 1 mM EDTA in PBS, washed three times with serum-free RPMI 1640, and re-suspended to an appropriate concentration in PBS. Syngeneic B6 mice were i.p. injected with 2 μg α-GalCer or vehicle (0.1% DMSO) on day −1 and then i.v. inoculated with B16 cells (5×10^4) on day 0. Some mice were primed with α-GalCer on day −4. Some mice were i.p. administered with 300 μg control rat Ig, anti-CD80 mAb, anti-CD86 mAb, and/or anti-B7RP-1 mAb 1.5 days before the last α-GalCer injection. On day 14, the number of tumor colonies in the lungs was counted under a dissecting microscope.

Statistical analysis. Data were analyzed by a two-tailed Student's *t* test. *P* values less than 0.05 were considered significant.

Results

ICOS expression on naïve and primed iNKT cells

Constitutive ICOS expression on iNKT cells was demonstrated by flow cytometric analysis of freshly isolated hapatic MNC (Fig. 1A) and splenic MNC (data not shown) from WT B6 mice. Although NK cells but not T cells constitutively expressed ICOS as reported [17], ICOS expression was more remarkable on iNKT cells compared to that on NK cells. ICOS expression was independent of CD28 since a similar level of ICOS expression was observed on iNKT cells isolated from $CD28^{-/-}$ mice, and CD28 expression was also independent of ICOS expression since a similar level of CD28 expression was observed on iNKT cells isolated from $ICOS^{-/-}$ mice (Fig. 1B). As ICOS was induced on conventional T cells by TCR ligation [6], ICOS expression on iNKT cells was up-regulated 2–3 days after α-GalCer treatment and then returned to the basal level by 5–7 days (Fig. 1C). Similar results were obtained with spleen MNC in vivo and after in vitro stimulation of liver MNC with α-GalCer (data not shown).

Involvement of ICOS costimulatory pathway in IFN-γ and IL-4 production by α-GalCer-stimulated iNKT cells

We next investigated whether iNKT cells require IOCS-mediated costimulation for producing cytokines

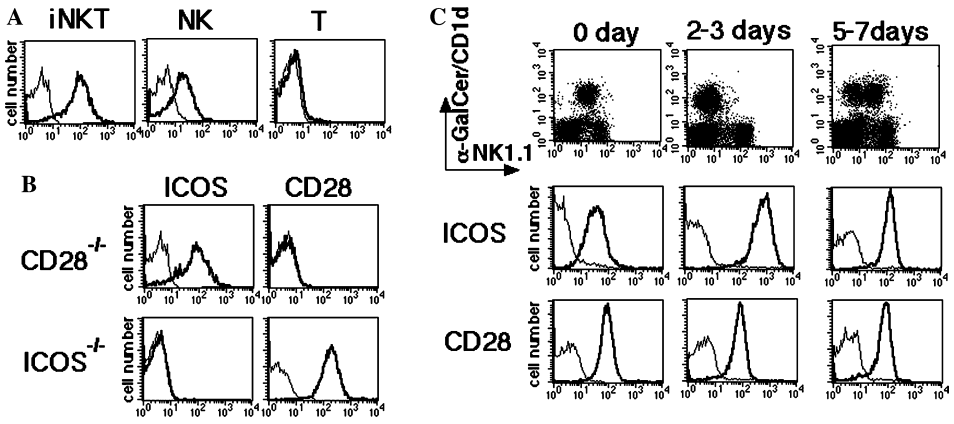

Fig. 1. ICOS expression on iNKT cells. (A) Surface expression of ICOS was analyzed on electronically gated α-GalCer/CD1d$^+$ iNKT cells, NK1.1$^+$ CD3$^-$ NK cells, and NK1.1$^-$CD3$^+$ T cells in liver MNC freshly isolated from WT B6 mice. (B) ICOS and CD28 expression on electronically gated α-GalCer/CD1d$^+$ iNKT cells in hepatic MNC freshly isolated from CD28$^{-/-}$ or ICOS$^{-/-}$ B6 mice. (C) ICOS and CD28 expression was analyzed on electronically gated α-GalCer/CD1d$^+$ iNKT cells on the indicated days after i.p. injection of α-GalCer. Thick lines indicate the staining with the respective mAb, and the thin lines indicate the staining with isotype-matched control Ig. Similar results were obtained from three independent experiments.

in response to α-GalCer. IFN-γ and IL-4 were detected in the supernatant of splenic MNC when cultured with α-GalCer for 48 h (Fig. 2A). Blockade of ICOS-mediated costimulation by anti-B7RP-1 mAb resulted in significant inhibition of both IFN-γ and IL-4 production, although blockade of CD28-mediated costimulation by anti-CD80 and anti-CD86 (anti-CD80/86) mAbs resulted in more remarkable but partial inhibition (Fig. 2A). Blockade of both ICOS- and CD28-mediated costimulation almost completely abolished IFN-γ and IL-4 production. The experiments using splenic MNC from ICOS$^{-/-}$ or CD28$^{-/-}$ mice also indicated the complementary roles of CD28 and ICOS in costimulation of iNKT cells (Fig. 2A). Consistent with the higher expression of ICOS on iNKT cells 3 days after α-GalCer priming (Fig. 1C), ICOS blockade more significantly inhibited IFN-γ and IL-4 production by splenic MNC from α-GalCer-primed mice 3 days before (Fig. 2A). IL-13 and IL-10 production was also inhibited in a similar manner and the analysis using supernatants after 72 h incubation demonstrated similar results (data not shown).

To exclude the possible contribution of T cells and NK cells to this α-GalCer-induced cytokine production, we performed a coculture of purified hepatic iNKT cells and purified splenic dendritic cells (DC). Both IFN-γ and IL-4 were detected in the culture supernatants when these cells were cocultured in the presence of α-GalCer for 48 h. This cytokine production was again partially inhibited by either anti-B7RP-1 mAb or anti-CD80/CD86 mAbs, and almost completely inhibited by the combination of these mAbs (Fig. 2B). This indicated that CD28 and ICOS were critical for iNKT cell activation by α-GalCer presented on DC. The analysis using supernatants after 72 h incubation demonstrated similar results (data not shown). Taken together, these results indicated that ICOS costimulated production not only of Th2 (IL-4, IL-10, and IL-13) but also Th1 (IFN-γ) cytokines by α-GalCer-stimulated iNKT cell independently of CD28 in vitro.

Contribution of ICOS to iNKT cell activation in vivo

To evaluate the contribution of IOCS to iNKT cell activation in vivo, we administered α-GalCer into WT mice following anti-B7RP-1 mAb and/or anti-CD80/86 mAb treatment. Blockade of either CD80/86–CD28 or B7RP-1–ICOS interaction partially reduced serum IFN-γ and IL-4 levels induced by α-GalCer injection, and blockade of both CD28 and ICOS pathways resulted in more remarkable reduction of both IFN-γ and IL-4 (Fig. 3A). Consistently, α-GalCer-induced serum IFN-γ and IL-4 levels were reduced in either ICOS$^{-/-}$ or CD28$^{-/-}$ mice compared to those in WT mice (Fig. 3B).

We next investigated the effect of ICOS blockade on α-GalCer-induced cytotoxic activity of splenic and hepatic MNC (Fig. 4). As previously reported [3,18,19], i.p. injection of α-GalCer into WT mice induced substantial cytotoxic activities of splenic and hepatic MNC against both NK-sensitive YAC-1 and NK-resistant P815 target cells. This induction of cytotoxic activities was partially inhibited by blockade of either CD80/86–CD28 or B7RP-1–ICOS interaction, and more remarkably reduced by blockade of both pathways. Consistently, α-GalCer-induced cytotoxic activity was reduced in either ICOS$^{-/-}$ or CD28$^{-/-}$ mice compared to that in WT mice, and treatment of CD28$^{-/-}$ mice with anti-B7RP-1 mAb or treatment of ICOS$^{-/-}$ mice with anti-CD80/86 mAbs further reduced the α-GalCer-induced cytotoxic activities (Fig. 4).

Then, we also examined the anti-metastatic effect of α-GalCer on experimental lung metastasis of B16

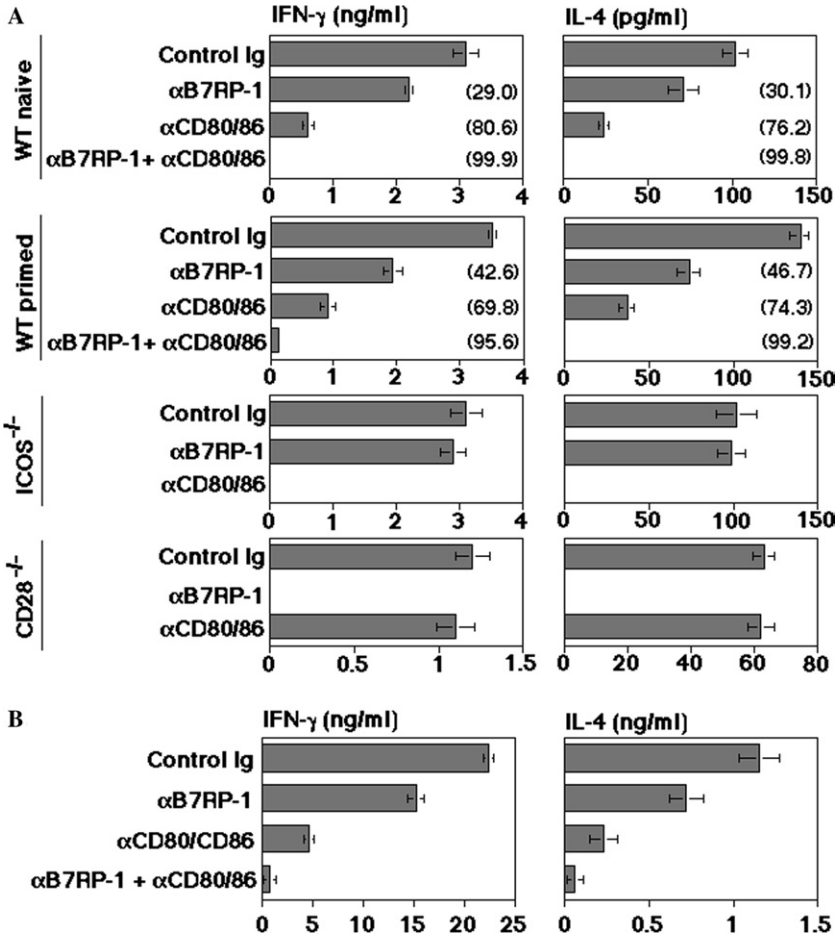

Fig. 2. CD28 and ICOS independently costimulate activation of iNKT cells by α-GalCer. (A) Splenic MNC were prepared from naïve WT, ICOS$^{-/-}$, or CD28$^{-/-}$ B6 mice, and WT B6 mice primed by i.p. injection of α-GalCer 3 days earlier. MNC were stimulated with α-GalCer in the presence of the indicated mAb for 48 h. IFN-γ and IL-4 levels in the cell-free supernatants were determined by ELISA. Percent inhibition compared to the culture with control Ig is indicated in parentheses. (B) Hepatic iNKT cells and splenic DC were separately isolated from naïve WT B6 mice, and then cocultured in the presence of α-GalCer and the indicated mAb for 48 h. IFN-γ and IL-4 levels in the cell-free supernatants were determined by ELISA. Data are represented as means ± SD of triplicate wells. Similar results were obtained from two independent experiments.

melanoma, which was mediated by iNKT cells [3,18,19] (Fig. 5). α-GalCer administration greatly reduced the number of lung metastatic nodules in WT mice. Treatment with either anti-B7RP-1 mAb or anti-CD80/86 mAbs significantly inhibited the α-GalCer-induced anti-metastasis effect, and the combination of these mAbs almost completely abolished the anti-metastatic effect of α-GalCer. Consistently, α-GalCer-induced anti-metastatic effect was substantially reduced in either ICOS$^{-/-}$ or CD28$^{-/-}$ mice compared to that in WT mice, and treatment of CD28$^{-/-}$ mice with anti-B7RP-1 mAb almost completely abolished the α-GalCer-induced anti-metastatic effect.

As expected from the FACS and in vitro analysis (Figs. 1C and 2), inhibitory effects of anti-B7RP-1 mAb treatment in α-GalCer-primed mice were more significant as compared to those in naïve mice on α-GalCer-induced serum cytokines (Fig. 3C), cytotoxic activity (Fig. 4), and anti-metastatic effect (Fig. 5). Taken together, these results indicated that ICOS was involved in the activation of both naïve and primed iNKT cells to exert their effector functions in vivo.

Discussion

In this study, we have shown that ICOS is constitutively expressed on naïve iNKT cells in a CD28-independent manner and further up-regulated by priming with α-GalCer. Consequently, interaction of ICOS with its ligand, B7RP-1, on APC costimulates naïve as well as primed iNKT cells to produce IFN-γ and IL-4 independently of CD28 in vitro. More importantly, we have demonstrated that the B7RP-1–ICOS interaction costimulates iNKT cells to exert their functions in vivo, inducing IFN-γ and IL-4 production, cytotoxic activity, and anti-metastatic activity. This is the first indication that ICOS is critically involved in iNKT cell activation.

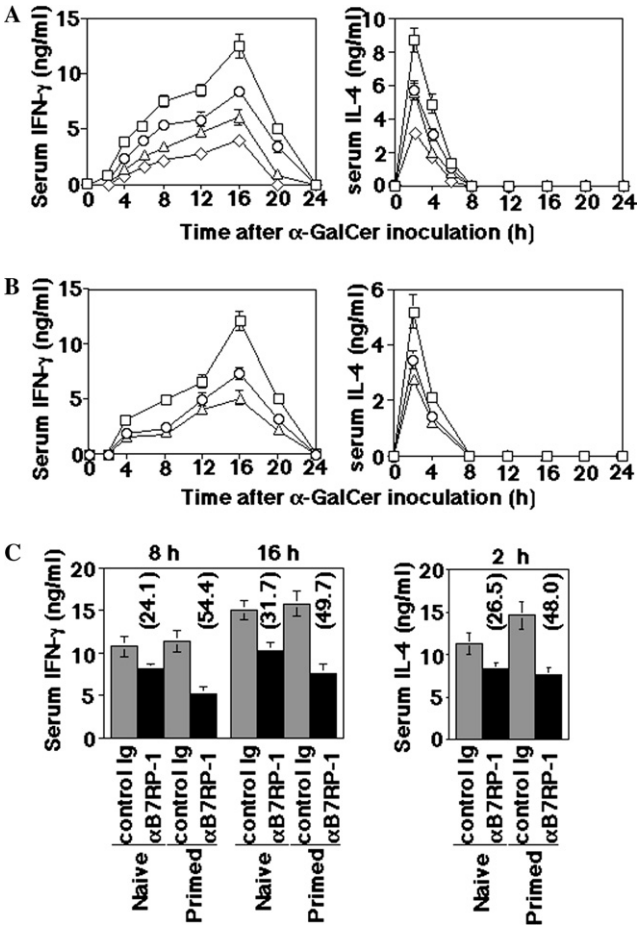

Fig. 3. Involvement of ICOS and CD28 in serum IFN-γ and IL-4 induction by α-GalCer. (A) Kinetics of serum IFN-γ and IL-4 levels after i.p. α-GalCer injection were analyzed in WT B6 mice. Mice were treated with control Ig (□), anti-B7RP-1 mAb (○), anti-CD80/CD86 mAbs (△), or anti-B7RP-1 mAb and anti-CD80/CD86 mAbs (◇). Data are represented as means ± SD of five mice in each group. Similar results were obtained from three independent experiments. (B) Kinetics of serum IFN-γ and IL-4 levels after i.p. α-GalCer injection were analyzed in B6 WT (□), ICOS$^{-/-}$ (○), or CD28$^{-/-}$ (△) B6 mice. Data are represented as means ± SD of five mice in each group. Similar results were obtained from three independent experiments. (C) Naïve or α-GalCer-primed (3 days before) WT B6 mice were i.p. injected with α-GalCer and control Ig or anti-B7RP-1 mAb. Serum IFN-γ and IL-4 levels were determined at the indicated time point. Percent inhibition compared to the control Ig-treated mice is indicated in parentheses. Data are represented as means ± SD of five mice in each group. Similar results were obtained from three independent experiments.

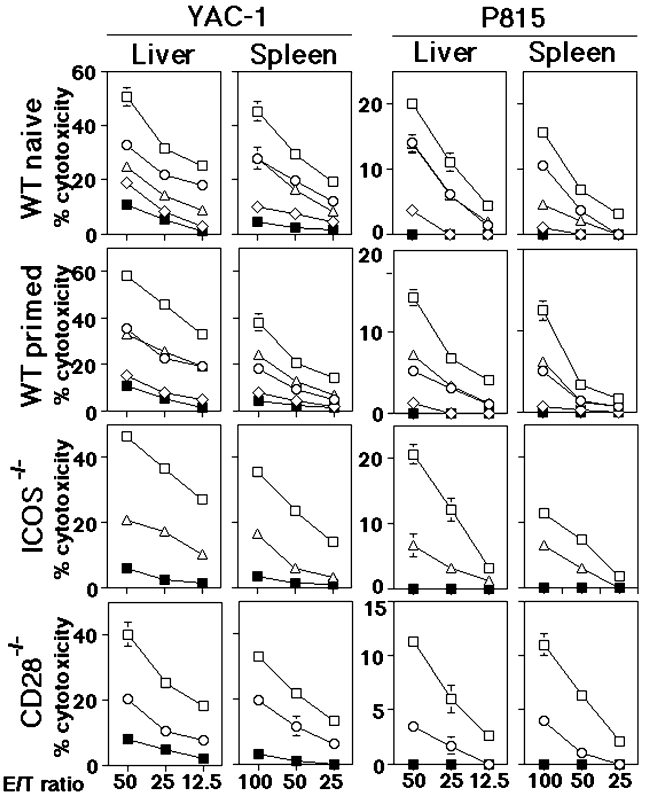

Fig. 4. Involvement of ICOS and CD28 in induction of cytotoxic activity by α-GalCer. Cytotoxic activities of liver and spleen MNC were tested against NK-sensitive YAC-1 and NK-resistant P815 target cells 24 h after α-Galcer injection into control Ig- (□), anti-B7RP-1 mAb- (○), anti-CD80/CD86 mAbs- (△), or anti-B7RP-1 mAb and anti-CD80/CD86 mAbs- (◇) treated mice. Cytotoxic activities of liver and spleen MNC isolated from untreated naïve or α-GalCer-primed (3 days before) mice are indicated as the control (■). Data are represented as means ± SD of triplicate samples. Similar results were obtained from three independent experiments.

It is notable that naïve iNKT cells express IOCS at a high level, while conventional T cells express IOCS only after activation [6]. This may be due to a pre-activated state of iNKT cells in naïve mice, because iNKT cells are potentially autoreactive and exhibit a memory-like phenotype as represented by CD44^{high} and CD62L^{low} [2]. Although it has been reported that optimal ICOS expression on conventional activated T cells was dependent on CD28-mediated costimulation [20], the ICOS expression on iNKT cells is not reduced in CD28-deficient mice. Further studies are needed to explore the mechanisms underlying the constitutive expression of IOCS on iNKT cells.

We have previously reported that CD28-mediated costimulation regulates both Th1- and Th2-type responses of iNKT cells, while CD40-mediated costimulatory pathway critically regulates Th1-type response of iNKT cells independently [3,5]. Moreover, it has been demonstrated that ICOS critically regulates CD154 expression on activated T cells [7–9], although ICOS delivers a costimulatory signal for IFN-γ, IL-4, and IL-10, but not IL-2, production independently of CD28 and CD40 [6]. Thus, ICOS costimulation might be mediated, at least in part, through the CD154–CD40 pathway in the regulation of Th1-type responses of iNKT cells. Further studies are now under way to reveal the correlation of ICOS and CD40 pathways.

While the blockade of either B7RP-1–ICOS or CD80/86–CD28 interaction only partly inhibited the production of IFN-γ and IL-4 by α-GalCer-stimulated

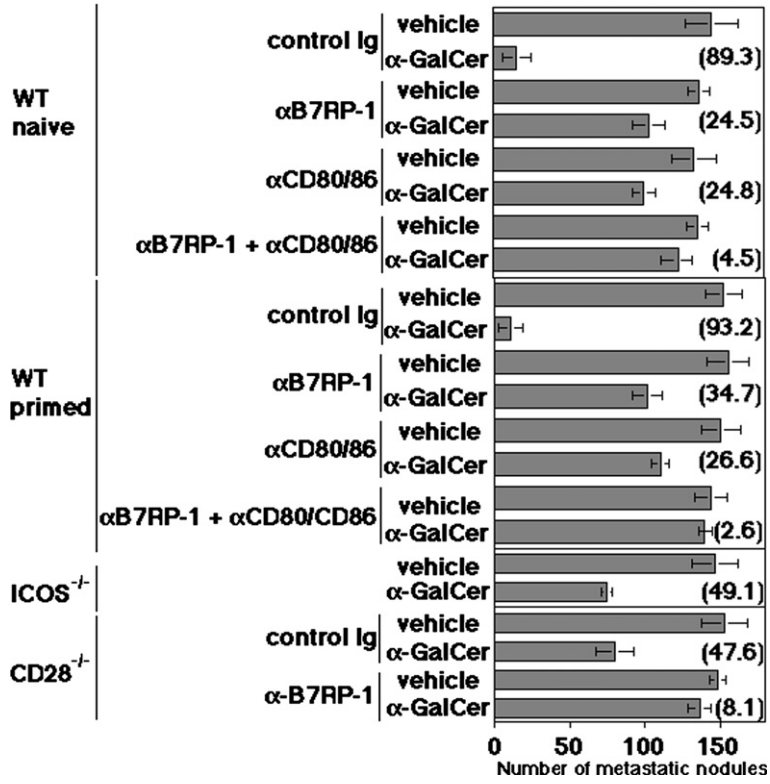

Fig. 5. Involvement of ICOS and CD28 in anti-metastatic effect of α-GalCer. Mice were i.p. injected with α-GalCer on day −1 and i.v. inoculated with 3×10^5 B16 melanoma on day 0. Some WT mice were i.p. primed with α-GalCer injection on day −4. Some mice were i.p. administered with control Ig or the indicated mAb on day −2.5. On day 14, the number of tumor metastatic colonies in the lungs was counted under a dissecting microscope. Data are represented as means ± SD of five mice in each group. Percent inhibition of metastasis compared to the vehicle-treated mice is indicated in parentheses. Similar results were obtained from three independent experiments.

splenic MNC, the blockade of both pathways almost completely abolished these responses in vitro. This indicates that these two pathways act complementarily and mostly account for costimulation by splenic APC, as represented by DC. In contrast, the IFN-γ and IL-4 production in response to α-GalCer administration in vivo was not completely abolished by the blockade of both ICOS and CD28 pathways, suggesting a possible contribution of other costimulatory molecules such as the members of TNF receptor family. Further studies are now under way to address this possibility.

It was initially reported that ICOS costimulation was preferentially involved in differentiation and function of Th2 cells [8,9]. However, recent studies have shown that ICOS also costimulates Th1 responses of conventional T cells [10,11]. We have now shown that ICOS costimulates production of both IFN-γ and IL-4 by iNKT cells. IFN-γ and IL-4 production by iNKT cells has been implicated in the pathogenesis of various diseases, such as hepatitis, abortion, and atherosclerosis [21–23]. Blockade of the ICOS costimulatory pathway would be more beneficial for amelioration of these diseases than blockade of the CD28 pathway, since ICOS plays rather predominant roles in the activation and function of effector T cells [7–9].

Acknowledgments

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References

[1] M. Kronenberg, L. Gapin, The unconventional lifestyle of NKT cells, Nat. Rev. Immunol. 2 (2002) 557–568.
[2] M. Taniguchi, M. Harada, S. Kojo, T. Nakayama, H. Wakao, The regulatory role of Vα14 NKT cells in innate and acquired immune response, Annu. Rev. Immunol. 21 (2003) 483–513.
[3] Y. Hayakawa, K. Takeda, H. Yagita, L. Van Kaer, I. Saiki, K. Okumura, Differential regulation of Th1 and Th2 functions of NKT cells by CD28 and CD40 costimulatory pathways, J. Immunol. 166 (2001) 6012–6018.
[4] Y. Ikarashi, R. Mikami, A. Bendelac, M. Terme, N. Chaput, M. Terada, T. Tursz, E. Angevin, F.A. Lemonnier, H. Wakasugi, L. Zitvogel, Dendritic cell maturation overrules H-2D-mediated natural killer T (NKT) cell inhibition: critical role for B7 in CD1d-dependent NKT cell interferon γ production, J. Exp. Med. 194 (2001) 1179–1186.
[5] H. Kitamura, K. Iwakabe, T. Yahata, S. Nishimura, A. Ohta, Y. Ohmi, M. Sato, K. Takeda, K. Okumura, L. Van Kaer, T. Kawano, M. Taniguchi, T. Nishimura, The natural killer T (NKT) cell ligand α-galactosylceramide demonstrates its

immunopotentiating effect by inducing interleukin (IL)-12 production by dendritic cells and IL-12 receptor expression on NKT cells, J. Exp. Med. 189 (1999) 1121–1128.
[6] A. Hutloff, A.M. Dittrich, K.C. Beier, B. Eljaschewitsch, R. Kraft, I. Anagnostopoulos, R.A. Kroczek, ICOS is an inducible T-cell co-stimulator structurally and functionally related to CD28, Nature 397 (1999) 263–266.
[7] C. Dong, A.E. Juedes, U.A. Temann, S. Shresta, J.P. Allison, N.H. Ruddle, R.A. Flavell, ICOS co-stimulatory receptor is essential for T-cell activation and function, Nature 409 (2001) 97–101.
[8] A.J. McAdam, R.J. Greenwald, M.A. Levin, T. Chernova, N. Malenkovich, V. Ling, G.L. Freeman, A.H. Sharpe, ICOS is critical for CD40-mediated antibody class switching, Nature 409 (2001) 102–105.
[9] A. Tafuri, A. Shahinian, F. Bladt, S.K. Yoshinaga, M. Jordana, A. Wakeham, L.M. Boucher, D. Bouchard, V.S. Chan, G. Duncan, B. Odermatt, A. Ho, A. Itie, T. Horan, J.S. Whoriskey, T. Pawson, J.M. Penninger, P.S. Ohashi, T.W. Mak, ICOS is essential for effective T-helper-cell responses, Nature 409 (2001) 105–109.
[10] E. Özkaynak, W. Gao, N. Shemmeri, C. Wang, J.C. Gutierrez-Ramos, J. Amaral, S. Qin, J.B. Rottman, A.J. Coyle, W.W. Hancock, Importance of ICOS-B7RP-1 costimulation in acute and chronic allograft rejection, Nat. Immunol. 2 (2001) 591–596.
[11] J.B. Rottman, T. Smith, J.R. Tonra, K. Ganley, T. Bloom, R. Silva, B. Pierce, J.C. Gutierrez-Ramos, E. Özkaynak, A.J. Coyle, The costimulatory molecule ICOS plays an important role in the immunopathogenesis of EAE, Nat. Immunol. 2 (2001) 605–611.
[12] R. liyama, T. Kanai, K. Uraushihara, T. Totsuka, T. Nakamura, T. Miyata, H. Yagita, A. Kushi, K. Suzuki, K. Tezuka, M. Watanabe, The role of inducible co-stimulator (ICOS)/B7-related protein-1 (B7RP-1) interaction in the functional development of Peyer's patches, Immunol. Lett. 88 (2003) 63–70.
[13] J.L. Matsuda, O.V. Naidenko, L. Gapin, T. Nakayama, M. Taniguchi, C.R. Wang, Y. Koezuka, M. Kronenberg, Tracking the response of natural killer T cells to a glycolipid antigen using CD1d tetramers, J. Exp. Med. 192 (2000) 741–754.
[14] S. Nuriya, H. Yagita, K. Okumura, M. Azuma, The differential role of CD86 and CD80 co-stimulatory molecules in the induction and the effector phases of contact hypersensitivity, Int. Immunol. 8 (1996) 917–926.
[15] H. Iwai, M. Abe, S. Hirose, F. Tsushima, K. Tezuka, H. Akiba, H. Yagita, K. Okumura, H. Kohsaka, N. Miyasaka, M. Azuma, Involvement of inducible costimulator-B7 homologous protein costimulatory pathway in murine lupus nephritis, J. Immunol. 171 (2003) 2848–2854.
[16] R. Maldonado-Lopez, T. De Smedt, P. Michel, J. Godfroid, B. Pajak, C. Heirman, K. Thielemans, O. Leo, J. Urbain, M. Moser, CD8α^+ and CD8α^- subclasses of dendritic cells direct the development of distinct T helper cells in vivo, J. Exp. Med. 189 (1999) 587–592.
[17] K. Ogasawara, S.K. Yoshinaga, L.L. Lanier, Inducible costimulator costimulates cytotoxic activity and IFN-γ production in activated murine NK cells, J. Immunol. 169 (2002) 3676–3685.
[18] T. Kawano, J. Cui, Y. Koezuka, I. Toura, Y. Kaneko, H. Sato, E. Kondo, M. Harada, H. Koseki, T. Nakayama, Y. Tanaka, M. Taniguchi, Natural killer-like nonspecific tumor cell lysis mediated by specific ligand-activated Vα14 NKT cells, Proc. Natl. Acad. Sci. USA 95 (1998) 5690–5693.
[19] Y. Hayakawa, K. Takeda, H. Yagita, S. Kakuta, Y. Iwakura, L. Van Kaer, I. Saiki, K. Okumura, Critical contribution of IFN-γ and NK cells, but not perforin-mediated cytotoxicity, to anti-metastatic effect of α-galactosylceramide, Eur. J. Immunol. 31 (2001) 1720–1727.
[20] A.J. McAdam, T.T. Chang, A.E. Lumelsky, E.A. Greefield, V.A. Boussiotis, J.S. Duke-Cohan, T. Chernova, N. Malenkovich, C. Jabs, V.K. Kuchroo, V. Ling, M. Collins, A.H. Sharpe, G.J. Freeman, Mouse inducible costimulatory molecule (ICOS) expression is enhanced by CD28 costimulation and regulates differentiation of CD4$^+$ T cells, J. Immunol. 165 (2000) 5035–5040.
[21] K. Takeda, Y. Hayakawa, L.V. Kaer, H. Matsuda, H. Yagita, K. Okumura, Critical contribution of liver natural killer T cells to a murine model of hepatitis, Proc. Natl. Acad. Sci. USA 97 (2000) 5498–5503.
[22] K. Ito, M. Karasawa, T. Kawano, T. Akasaka, H. Koseki, Y. Akutsu, E. Kondo, S. Sekiya, K. Sekikawa, M. Harada, M. Yamashina, T. Nakayama, M. Taniguchi, Involvement of decidual Vα14 NKT cells in abortion, Proc. Natl. Acad. Sci. USA 97 (2000) 740–744.
[23] E. Tupin, A. Nicoletti, R. Elhage, M. Rudling, H. Ljunggren, G.K. Hasson, G.P. Berne, CD1d-dependent activation of NKT cells aggravated atherosclerosis, J. Exp. Med. 199 (2004) 417–422.