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Th1 or Th2 Balance Regulated by Interaction between Dendritic Cells and NKT Cells

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Short title: Feedback regulation of Th1/Th2 balance

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Abstract

If Th1 or Th2 polarization could be artificially manipulated, effective immune responses would be generated depending on nature of the targets. In this study we attempted to regulate CD40 expressions on dendritic cells (DCs) in order to modify the T cell response. It was found that reducing agents selectively inhibited surface expression of CD40 on DCs. This finding may provide a new strategy of DC-mediated modulation of the Th1/Th2 balance. It was also shown that NKT-produced Th1/Th2 cytokine balance was under control of negative feedback loop through DCs. Th1 cytokine-pretreated DCs mainly induced Th2 cytokine production, whereas Th2 cytokine-pretreated DCs induced Th1 cytokine production by α -galactosylceramide-stimulated NKT cells. The negative feedback regulation system could be applicable to therapeutics of various diseases based on immunological disorders.

Introduction

Immunological responses can be divided into two types, T helper 1 (Th1) and Th2. In the Th1 system, interferon (IFN)- γ , interleukin (IL-2) and tumor necrosis factor (TNF)- α play a major role in protective immunity against viruses, bacteria and tumors (Table 1). However, the Th1 immunity sometimes aggravates certain types of autoimmune diseases and atherosclerosis (1-3). On the other hand, IL-4, IL-10 and IL-13 play a role in Th2 type immunity. The Th2 system is important in anti-parasite reaction, and regulates autoimmune diseases, but aggravates allergy (4, 5).

Naive T cells differentiate into Th1 or Th2 cells, which are influenced by the environmental condition around these helper T cells. For instance, IL-12 produced by dendritic cells (DCs) promotes Th1 differentiation (6), whereas IL-4 produced by Th2 cells favors induction of Th2 cells in an autocrine manner and establishes Th2 polarization (4, 7). We also reported that TNF- α enhanced preference of DCs for Th2 differentiation (8). The Th1 or Th2 type determines effectiveness of the response, depending on nature of the targets. Thus, if Th1 or Th2 polarization could be artificially manipulated, we can generate effective responses.

In this brief review we will show a manipulation to regulate CD40 expressions on dendritic cells, which may result in modification of the type of T cell responses (8 - 10). Then, we report a novel regulation system governing the Th1/Th2 cytokine balance via DC and natural killer T (NKT) cell interaction (11,12).

Modification of a Costimulatory Molecule, CD40, on DCs

DCs are most potent antigen presenting cells (13, 14). Immature DCs vigorously capture antigens (Fig. 1). After stimulation with bacterial products or inflammatory cytokines, these immature DCs mature. Mature DCs then migrate to lymph nodes in the presence of chemokines, CCL19 and CCL21. These mature DCs express high levels of major

histocompatibility complexes (MHC) and costimulatory molecules to present antigens. DCs are the only cells that can stimulate naive T cells. DCs also express high levels of non-polymorphic CD1d, a MHC class Ib molecule, and stimulate NK-T cells.

Figure 2 shows a representative morphology of immature and mature DCs. The large size mature DCs possess long dendrites compared to immature DCs. We have reported that CCL19 chemokine induces not only chemotaxis but also dendrite extension and endocytosis in mature DCs (15, 16). The CCL19-induced endocytosis and migration of mature DCs are mediated by different signal pathways (17). MHC class II molecules are seen in the cytoplasm of immature DCs (Fig. 2). By contrast, the MHC class II molecules are expressed on the cell membrane of mature DCs.

Various surface molecules are expressed on DCs (Fig. 3). These MHC and costimulatory molecules on the DC and T cell antigen receptor (TCR) and costimulatory molecules on the T cell are indispensable for full T cell activation (Fig. 3). Especially, stimulation of CD40 on the DC by CD40 ligand on the T cell results in promotion of IL-12 production by the DC and Th1 shift. Thus, if CD40 expression on the DCs could be regulated, the Th1 polarization may be prevented.

Reduction of CD40 Expression on DCs

We could successfully established a stable immature DC line (BC1 cells) (18) and, using this cell line, we attempted to reduce the CD40 expression on the DCs. Eventually we found that anti-oxidants such as N-acetyl-L-cysteine (NAC) selectively reduced the expression of CD40 on DCs (9). As shown in Figure 4, TNF- α upregulated CD80, 86 and 40 and MHC class I and II expressions. When these DCs were further treated with NAC, only CD40 expression was markedly and selectively reduced by the NAC.

The anti-oxidant-mediated reduction of CD40 expression was attributed to the decrease of

total CD40 protein in the mature DCs. The TNF- α induced expression of total CD40 protein was significantly reduced by NAC (Fig. 5). However, no reduction of CD40 mRNA was induced by the NAC. Similar effects were observed when reduced glutathione, a physiological counterpart of NAC, was used to treat DCs (data not shown) (9). These findings suggest that the anti-oxidant affects a post-transcriptional pathway such as translation and/or degradation of CD40 protein.

Cytokine Production of DCs Stimulated through CD40

We then analyzed NF- κ B activation and IL-6 and IL-12 productions by DCs stimulated through CD40. In this particular experiment DCs established using *aly/aly* mice and the *aly/+* heterozygotes (C57BL/6 background) were analyzed. Increased levels of phosphorylated p100, an alternative NF- κ B pathway member, were seen in mature DCs after stimulation with anti-CD40 monoclonal antibody (mAb) (19) (Fig. 6). It was also shown in the Figure 6B that the CD40 stimulation induced prominent production of IL-6 and IL-12 in DCs of normal *aly/+* heterozygotes. However, the CD40-mediated IL-12 production was very low in DCs of *aly/aly* mouse. The *aly/aly* mouse lacks phosphorylation of p100, because of a mutation of NF- κ B inducing kinase (20). Thus, these findings suggest that NF- κ B pathway is involved in IL-12 production by CD40-stimulated DCs.

In the next experiment we analyzed relationship between activation of p38 MAP kinase and cytokine production in CD40-stimulated DCs (BC1 cells). Treatment with anti-CD40 mAb activated p38 MAPK and induced prominent IL-6 and IL-12 productions in mature DCs (Fig. 7) as was shown in Figure 6. It was also demonstrated in Figure 7B that the IL-6, but not IL-12 production was significantly reduced by SB 203580, a p38 MAPK inhibitor. This finding suggests that the p38 MAPK activation is involved in IL-6 but not IL-12 production by mature DCs. From these findings we conclude that IL-6 and IL-12 production by CD40-stimulated

mature DCs is controlled by p38 MAPK and NK- κ B pathway, respectively (Fig. 8).

DC-Mediate Modification of Immunological Responses (Summary 1)

Table 2 summarizes the first part of this review. DCs express surface molecules for Ag presentation along with maturation. Especially CD40 is important in Th1 polarization. Reducing agents such as N-acetyl-L-cysteine and reduced glutathione selectively inhibit surface expression of CD40 by regulating the post-transcriptional pathway.

NF- κ B activation is involved in the CD40-mediated IL-12 production and p38 MAPK in IL-6 production by mature DCs. Thus, the CD40 stimulation, IL-12 production and Th1 shift story seems not to be that simple. Nevertheless, we consider that these findings may be the basis of DC-mediated modulation of the Th1/Th2 balance.

Negative Feedback Regulation of Th1/Th2 Balance via DC and NKT Cell Interactions

NKT cells are a unique subset of T cells that express both NK receptors and limited T cell receptors (TCR) (21). In human case, the expression of V α 24 and V β 11 is frequently used to identify NKT cells (12) (Fig. 9). These NKT cells recognize lipid antigens in the context of non-polymorphic CD1d molecules and produce very quickly large amounts of various cytokines upon stimulation (2, 7, 22, 23). NKT cells kill not only tumor cells but also self immature thymocytes via Fas pathway (24, 25).

A major population of murine NKT cell expresses an invariant V α chain, V α 14J α 18, paired with a restricted V β chains (Fig. 9) (21, 26). However, we have reported heterogenous subpopulations among NKT cells (27, 28). In particular TCR transgenic mice, NKT cells with typical NKT characteristics are present in T cell population expressing TCR other than V α 14J α 18. In addition, NKT cells undergo a unique development different from that of mainstream T cells (29, 30). NKT cells are positively selected by intrinsic lipid antigens plus

CD1d expressed on CD4, 8 double positive thymocytes under the influence of medullary thymic epithelial cells (30, 31).

Recently it has been generally accepted that atherosclerosis is developed under the influence of not only hyperlipidemia but also inflammatory cells including macrophages and Th1 cells (32, 33). We have reported that NK-T cells responding to oxidized low-density lipoprotein (LDL), produce IFN- γ and aggravates atherosclerosis (3). It is shown in Figure 9 bottom panel that NKT cells expressing V α 14 mRNA are present in the atherosclerotic lesion but not in normal arterial wall.

In Vitro Stimulation of NKT Cells by α -galactosylceramide (α -GalCer)

NKT cells recognize α -GalCer, which is derived from a marine sponge (34), presented by CD1d on DCs and quickly produce a huge amount of IFN- γ and IL-4 (Fig. 10). We attempted to modulate DC functions in order to control the type of cytokine production by NKT cells.

Dendritic cells were prepared from BALB/c splenocytes as previously described (11, 18). These CD11c positive DCs were then loaded with α -GalCer for 1 day and treated with IL-4 or IFN- γ for another 3 days. Subsequently, these α -GalCer-loaded DCs were cultured with nylon-nonadherent splenocytes, a source of NKT cells, from either BALB/c or CD1d knockout (KO) mice of BALB/c background for 48 hours and cytokine levels in the culture supernatant were quantitated by enzyme-linked immunosorbent assay (ELISA).

As shown in Figure 11A left panel, IL-4-pretreated DCs induced significantly higher IFN- γ production by NKT cells than IFN- γ pretreated DCs or phosphate buffered saline (PBS)-treated immature DCs. In contrast, as shown in the Figure 11A right panel, IFN- γ pretreated DCs induced considerably greater IL-4 production than immature DCs or IL-4-pretreated DCs. No cytokine production was seen at all in splenocytes from CD1d KO mice where invariant NKT cells responding to α -GalCer are absent (Fig. 11B).

In Vivo Stimulation of NKT Cells by α -GalCer-Loaded DCs

Then, to analyze ability of each DC group to stimulate NKT cells *in vivo*, we injected each type of α -GalCer-loaded DCs into mouse spleens and sequentially examined IL-4 and IFN- γ levels in the serum. It has been reported that α -GalCer-loaded DCs efficiently present α -GalCer to NKT cells and generate a long-lasting responses (35, 36).

Figure 12 shows the serum levels of IL-4 and IFN- γ in mice given α -GalCer-loaded DCs at 6 h and 12 h (maximum response), respectively. The IL-4 pretreated DCs induced a significantly higher amount of IFN- γ than immature DCs or IFN- γ pretreated DCs. By contrast, IFN- γ pretreated-DCs induced significantly higher production of IL-4 than immature DCs or IL-4 pretreated-DC.

We then analyzed *in vivo* effects of both IL-4 and α -GalCer administration. BALB/c and CD1d KO mice were given PBS or IL-4 and 3 days later administered α -GalCer. To protect IL-4 from rapid degradation *in vivo*, we used IL-4 complex (IL-4C), a complex of IL-4 with anti-IL-4 antibody, in *in vivo* experiment (37). After administration of IL-4 and α -GalCer the serum cytokine level was sequentially measured. Figure 13A shows serum IFN- γ level in mice given α -GalCer administration. The pretreatment with IL-4 significantly enhanced IFN- γ production in sera of α -GalCer-injected mice as compared with mice treated with α -GalCer alone.

Cytotoxic Activity of Splenocytes from IL-4 and α -GalCer-Administered Mice

We next analyzed cytotoxic activity of splenocytes from IL-4C-pretreated mice against various tumor cells 24 hours after α -GalCer administration. It will be seen in Figure 13B that administration of α -GalCer alone induced considerable cytotoxicity against tumor cells and this α -GalCer-induced cytotoxicity was markedly augmented by the IL-4 pretreatment.

In the last experiment, we evaluated effect of IL-4 pretreatment on the α -GalCer-induced

anti-cancer activity using a lung metastasis model. BALB/c mice were pretreated with IL-4C, and 3 days later intravenously injected with renal adenocarcinoma cells together with α -GalCer (Fig. 14). Three weeks later, the number of lung metastases was counted.

Figure 15B shows a representative view of lung metastasis, and Figure 15A shows the mean metastasis number. Treatment with α -GalCer alone markedly inhibited metastasis as previously reported (35, 36, 38).

It should be noted here that significantly reduced metastases were observed in mice treated with both IL-4 and α -GalCer compared with those in mice treated with α -GalCer alone. No anti-metastatic effect was observed in mice given PBS or IL-4C alone (Fig. 15). These findings demonstrate that treatment of mice with IL-4 and α -GalCer markedly enhances IFN- γ production and anti-tumor responses.

Negative Feedback Regulation between DCs and NKT Cells (Summary 2)

Figure 16 summarizes the second part of this review. NKT-produced Th1/Th2 cytokine balance is under control of negative feedback loop through DCs. Th1 cytokine-pretreated DCs mainly induced Th2 cytokine production, whereas Th2 cytokine-pretreated DCs induced Th1 cytokine production in NKT cells.

We also showed that in vivo IL-4 pretreatment of mice led to augmented anti-cancer effect by an NKT ligand, α -GalCer. We hope that the negative feedback regulation system with the use of NKT-specific ligands could be applicable to therapeutics of not only cancer, but also various infectious diseases, and autoimmune diseases (5, 11, 23, 39, 40).

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References

1. Stockinger B, Bourgeois C, Kassiotis G: CD4 memory T cells: functional differentiation and homeostasis. *Immunol Rev* 2006; 211: 39-48.
2. Diao H, Kon S, Iwabuchi K, Kimura C, Morimoto J, Ito D, Segawa T, Maeda M, Hamuro J, Nakayama T, Taniguchi M, Yagita H, Van Kaer L, Onoé K, Denhardt D, Rittling S, Uede T: Osteopontin as a mediator of NKT cell function in T cell mediated liver diseases. *Immunity* 2004; 21(4): 539-550.
3. Nakai Y, Iwabuchi K, Fujii S, Ishimori N, Watano K, Mishima T, Iwabuchi C, Tanaka S, Dashtsoodol N, Nakayama T, Taniguchi M, Miyake S, Yamamura T, Kitabatake A, Joyce S, Van Kaer L, Onoé K: Natural killer T cells accelerate atherogenesis in mice. *Blood* 2004; 104(7): 2051-2059.
4. Hochrein H, O'Keeffe M, Luft T, Vandenabeele S, Grumont RJ, Maraskovsky E, Shortman K: Interleukin (IL)-4 is a major regulatory cytokine governing bioactive IL-12 production by mouse and human dendritic cells. *J Exp Med* 2000; 192(6): 823-833.
5. Miyamoto K, Miyake S, Yamamura T: A synthetic glycolipid prevents autoimmune encephalomyelitis by inducing TH2 bias of natural killer T cells. *Nature* 2001; 413(6855): 531-534.
6. Macatonia SE, Hosken NA, Litton M, Vieira P, Hsieh CS, Culpepper JA, Wysocka M, Trinchieri G, Murphy KM, O'Garra A: Dendritic cells produce IL-12 and direct the development of Th1 cells from naive CD4⁺ T cells. *J Immunol* 1995; 154(10):5071-5079.
7. Yoshimoto T, Bendelac A, Hu-Li J, Paul WE: Defective IgE production by SJL mice is linked to the absence of CD4⁺, NK1.1⁺ T cells that promptly produce interleukin 4. *Proc Natl Acad Sci USA* 1995; 92(25):11931-11934.
8. Kikuchi K, Yanagawa Y, Aranami T, Iwabuchi C, Iwabuchi K, Onoé K: Tumour necrosis

- factor- α but not lipopolysaccharide enhances preference of murine dendritic cells for Th2 differentiation. *Immunology* 2003; 108(1): 42-49.
9. Iijima N, Yanagawa Y, Iwabuchi K, Onoé K: Selective regulation of CD40 expression in murine dendritic cells by thiol antioxidants. *Immunology* 2003; 110(2): 197-203.
 10. Iijima N, Yanagawa Y, Onoé K: Role of early and late phase activation of p38 MAPK induced by TNF- α or 2, 4-dinitrobenzene during maturation of dendritic cells. *Immunology* 2003; 110(3): 322-328.
 11. Minami K, Yanagawa Y, Iwabuchi K, Shinohara N, Harabayashi T, Nonomura K, Onoé K: Negative feed back regulation of T helper type 1 (Th1)/Th2 cytokine balance via dendritic cell and natural killer T cell interactions. *Blood* 2005; 106(5): 1685-1693.
 12. Takagi D, Iwabuchi K, Iwabuchi C, Nakamaru Y, Maguchi S, Ohwatari R, Furuta Y, Fukuda S, Joyce S, Onoé K: Immunoregulatory defects of V α 24⁺V β 11⁺ NKT cells in development of Wegener's granulomatosis and relapsing polychondritis. *Clin Exp Immunol* 2004; 136(3): 591-600.
 13. Steinman RM: The dendritic cell system and its role in immunogenicity. *Annu Rev Immunol* 1991; 9: 271-296.
 14. Banchereau J, Steinman RM: Dendritic cells and the control of immunity. *Nature* 1998; 392 (6673): 245-251.
 15. Yanagawa Y, Onoé K: CCL19 induces rapid dendritic extension of murine dendritic cells. *Blood* 2002; 100(6): 1948-1956.
 16. Yanagawa Y, Onoé K: CCR7 ligands induce rapid endocytosis in mature dendritic cells with concomitant up-regulation of Cdc42 and Rac activities. *Blood* 2003; 101(12): 4923-4929.
 17. Iijima N, Yanagawa Y, Clingan JM, Onoé K: CCR7-mediated c-Jun N-terminal kinase activation regulates cell migration in mature dendritic cells. *Int Immunol* 2005; 17(9):

1201-1212.

18. Yanagawa Y, Iijima N, Iwabuchi K, Onoé K: Activation of extracellular signal-related kinase by TNF- α controls the maturation and function of murine dendritic cells. *J Leukoc Biol* 2002; 71(1): 125-132.
19. Yanagawa Y, Onoé K: Distinct regulation of CD40-mediated interleukin (IL)-6 IL-12 production via mitogen-activated protein kinase (MAPK) and nuclear factor κ B kinase (NIK) in mature dendritic cells. *Immunology* 2006; 117(4): 526-535.
20. Shinkura R, Kitada K, Matsuda F, Tashiro K, Ikuta K, Suzuki M, Kogishi K, Serikawa T, Honjo T: Alymphoplasia is caused by a point mutation in the mouse gene encoding NF- κ B-inducing kinase. *Nat Genet* 1999; 22(1): 74-77.
21. Arase H, Arase N, Ogasawara K, Good RA, Onoé K: An NK1.1⁺ CD4⁺8⁻ single positive thymocyte subpopulation that expresses a highly skewed T-cell antigen receptor V β family. *Proc Natl Acad Sci USA* 1992; 89(14): 6506-6510.
22. Arase H, Arase N, Nakagawa K, Good RA, Onoé K: NK1.1⁺ CD4⁺ CD8⁻ thymocytes with specific lymphokine secretion. *Eur J Immunol* 1993; 23(1): 307-310.
23. Terabe M, Matsui S, Noben-Trauth N, Chen H, Watson C, Donaldson DD, Carbone DP, Paul WE, Berzofsky JA: NKT cell-mediated repression of tumor immunosueveillance by IL-13 and the IL-4-STAT 6 pathway. *Nat Immunol* 2000; 1(6): 515-520.
24. Arase H, Arase N, Kobayashi Y, Nishimura Y, Yonehara S, Onoé K: Cytotoxicity of fresh NK1.1⁺ T-cell receptor α β ⁺ thymocytes against a CD4⁺8⁺ thymocyte population associated with intact Fas antigen expression on the target. *J Exp Med* 1994; 180(2): 423-432.
25. Arase H, Arase-Fukushi N, Good RA, Onoé K: Lymphokine activated killer cell activity of CD4⁻CD8⁻ TCR α β ⁺ thymocytes. *J Immunol* 1993; 151(2): 546-555.

26. Makino Y, Kanno R, Koseki H, Taniguchi M: Development of V α 14⁺ NK T cells in the early stages of embryogenesis. *Proc Natl Acad Sci USA* 1996; 93(13):6516-6520.
27. Iwabuchi C, Iwabuchi K, Nakagawa K, Takayanagi T, Nishihori H, Tone S, Ogasawara K, Good R A, Onoé K: Intrathymic selection of NK1.1⁺ α/β T cell antigen receptor (TCR)⁺ cells in transgenic mice bearing TCR specific for chicken ovalbumin and restricted to I-A^d. *Proc Natl Acad Sci USA* 1998; 95(14): 8199-8204.
28. Ohwatari R, Iwabuchi K, Iwabuchi C, Morohashi T, Sawa H, Hioki K, Kobayashi K, Fukuda S, Inuyama Y, Onoé K: Developmental and functional analyses of CD8⁺ NK1.1⁺ T cells in the class I restricted TCR transgenic mice. *Cell Immunol* 2001; 213(1):24-33.
29. Iwabuchi K, Iwabuchi C, Tone S, Itoh D, Tosa N, Negishi I, Ogasawara K, Uede T, Onoé K: Defective development of NK1.1⁺ T cell antigen receptor $\alpha\beta$ ⁺ cells in zeta-associated protein 70 null mice with an accumulation of NK1.1⁺ CD3⁻ NK-like cells in the thymus. *Blood* 2001; 97(11):1765-1775.
30. Nakagawa K, Iwabuchi K, Ogasawara K, Ato M, Kajiwara M, Nishihori H, Iwabuchi C, Ishikura H, Good RA, Onoé K: Generation of NK1.1⁺ TCR α / β ⁺ thymocytes associated with intact thymic structure. *Proc Natl Acad Sci USA* 1997; 94(6): 2472-2477.
31. Konishi J, Iwabuchi K, Iwabuchi C, Ato M, Nagata J, Onoé K, Nakagawa K, Kasai M, Ogasawara K, Kawakami K, Onoé K: Thymic epithelial cells responsible for impaired generation of NK-T thymocytes in *alymphoplasia* mutant mice. *Cell Immunol* 2000; 206(1): 26-35.
32. Ross R: Atherosclerosis-an inflammatory disease. *N Engl J Med* 1999; 340(2): 115-126.
33. Ishimori N, Iwabuchi K, Fujii S, Watano K, Iwabuchi C, Ato M, Chiba H, Kitabatake A, Onoé K: Mixed allogeneic chimerism with wild-type strains ameliorated atherosclerosis in apolipoprotein E-deficient mice. *J Leukoc Biol* 2001; 69(5): 732-740.
34. Kawano T, Cui J, Koezuka Y, Toura I, Kaneko Y, Motoki K, Ueno H, Nakagawa R, Sato

- H, Kondo E, Koseki H, Taniguchi M: CD1d-restricted and TCR-mediated activation of V α 14 NKT cells by glycosylceramides. *Science* 1997; 278(5343): 1626-1629.
35. Fujii S, Shimizu K, Kronenberg M, Steinman RM: Prolonged IFN- γ -producing NKT response induced with α -galactosylceramide-loaded DCs. *Nat Immunol* 2002; 3(9): 867-874.
 36. Chang DH, Osman K, Connolly J, Kukreja A, Krasovsky J, Pack M, Hutchinson A, Geller M, Liu N, Annable R, Shay J, Kirchhoff K, Nishi N, Ando Y, Hayashi K, Hassoun H, Steinman RM, Dhodapkar MV: Sustained expansion of NKT cells and antigen-specific T cells after injection of α -galactosyl-ceramide loaded mature dendritic cells in cancer patients. *J Exp Med* 2005; 201(9):1503-1517.
 37. Finkelman FD, Madden KB, Morris SC, Holmes JM, Boiani N, Katona IM, Maliszewski CR: Anti-cytokine antibodies as carrier proteins. Prolongation of in vivo effects of exogenous cytokines by injection of cytokine-anti-cytokine antibody complexes. *J Immunol* 1993; 151(3):1235-1244.
 38. Smyth MJ, Crowe NY, Pellicci DG, Kyparissoudis K, Kelly JM, Takeda K, Yagita H, Godfrey DI: Sequential production of interferon- γ by NK.1.1 (+) T cells and natural killer cells is essential for the antimetastatic effect of α -galactosylceramide. *Blood* 2002; 99(4):1259-1266.
 39. Wilson M T, Singh A K, Van Kaer L: Immunotherapy with ligands of natural killer T cells. *Trends Mol Med* 2002; 8(5):225-231.
 40. Taniguchi M, Harada M, Kojo S, Nakayama T, Wakao H: The regulatory role of V α 14 NKT cells in innate and acquired immune response. *Annu Rev Immunol* 2003; 21: 483-513.

Table 1 Two Aspects of Th1 and Th2 Responses

Th1 response	1. Mediated by IFN- γ , IL-2, TNF- α
(cellular)	2. Generates protective responses against viruses, bacteria and tumor cells
	3. Aggravates certain types of autoimmune diseases and atherosclerosis

Th2 response	1. Mediated by IL-4, IL-10, IL-13
(humoral)	2. Generates protective responses against parasites
	3. Regulates unfavorable responses (i.e. autoimmune diseases)
	4. Aggravates allergy

Tbale 2 functional Manipulation of DCs

1. DCs express costimulatory molecules (i.e. CD40) as well as MHC and CD1d molecules during maturation.
2. NAC and reduced glutathione selectively inhibit surface expression of CD40 protein on mature DCs by regulating the post-transcriptional pathway (with no influence on ERK, p38 MAPK and SAPK/JNK activities).
3. Stimulation via CD40 leads to IL-12 production through NF- κ B pathway, and IL-6 production through p38 MAPK pathway in mature DCs, which may be involved in Th1 or Th2 polarization.

These findings may be the basis of DC-mediated modulation of the Th1/Th2 balance.

Figure Legends

Fig. 1. Functional maturation of dendritic cells (DCs).

Fig. 2. Morphologic change from immature DCs to mature DCs. MHC class II expression is indicated by red color.

Fig. 3 Various surface molecules involved in interaction between DCs and T cells. Stimulatory signals through CD40 enhance not only maturation of DCs but also IL-12 production.

Fig. 4 Effects of NAC on expression of MHC and costimulatory molecules on DCs (Ref. 9)

Fig. 5. CD40 expression in DC line stimulated with TNF- α . Total CD40 protein (A) and mRNA (B) expression in DCs treated with TNF- α in the presence of NAC are shown (Ref. 9).

Fig. 6. NF- κ B activation and cytokine production following stimulation through CD40. DCs derived from *aly/+* and *aly/aly* NF- κ B inducing kinase (NIK) mutant mice were analyzed (Ref. 19). A. NF- κ B is activated by anti-CD40 mAb in mature DCs. B. IL-6 and IL-12 production by DCs stimulated through CD40. NF- κ B activation is involved in IL-12 but not IL-6 production in DCs. ** $p < 0.01$, * $p < 0.05$

Fig. 7. p38 MAPK activation and cytokine production. BC1 cells treated with LPS were used as mature DCs (Ref. 19). A. p38 is activated in both immature and mature DCs. B. IL-6 and IL-12 production. SB203580, a p38 MAPK inhibitor, reduced IL-6 but not IL-12

production in mature DC line. * $p < 0.05$

Fig. 8. IL-6 or IL-12 production in response to CD40 ligation controlled by p38 MAPK or NIK-mediated NF- κ B alternative pathway, respectively in mature DCs.

Fig. 9. Characteristics of NKT cells. An atherosclerotic arterial wall was obtained from apolipoprotein (apo) E KO mice (left bottom panel). Expression of V α 14 J α 18 mRNA was analyzed by RT-PCR (right bottom panel) (Ref. 3). Note the prominent expression of V α 14 mRNA in the atherosclerotic lesion, whereas no expression of the V α 14 in normal arterial wall.

Fig. 10. NKT cell recognizes glycolipid antigen, α -GalCer, in the context of CD1d and produce a copious amount of cytokines.

Fig. 11. IFN- γ and IL-4 productions by NKT cells stimulated with α -GalCer-loaded DCs pretreated with IFN- γ or IL-4 (Ref. 11). A. BALB/c splenocytes were stimulated with α -GalCer-loaded DCs. B. CD1d KO splenocytes (lacking iNKT cells) were stimulated with α -GalCer-loaded DCs. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$

Fig. 12. Serum IFN- γ and IL-4 levels in BALB/c mice received cytokine-pretreated α -GalCer-loaded DCs. DCs (5×10^5) treated as described in the Fig. 11 legend were injected into the mouse spleen and IL-4 and IFN- γ levels in the serum were sequentially examined (Ref. 11). * $p < 0.05$

.Fig. 13. Effects of IL-4 administration on NKT cell responses (Ref. 11). A. IFN- γ level in

serum of IL-4- pretreated and α -GalCer- administrated mice. BALB/c and CD1d KO mice (BALB/c background) were given PBS or IL-4 and 3 d later administered α -GalCer. Thereafter, serum IFN- γ level was sequentially quantitated. B. Cytotoxic activity of splenocytes from IL-4-pretreated and α -GalCer- administrated mice. ** $p < 0.01$, * $p < 0.05$

Fig. 14. Anti-cancer effect of IL-4 plus α -GalCer administration using a lung metastasis model. BALB/c mice were pretreated with IL-4C and 3 d later intravenously injected with RenCa cells (5×10^5) and α -GalCer (2 μ g/head). Three weeks later, the number of lung metastasis was counted.

Fig. 15. Lung metastasis of RenCa cells in IL-4-pretreated and α -GalCer- administrated BALB/c mice (Ref. 11). A. Mean number of lung metastasis $\pm$ SE. B. Representative view of lung metastasis. ** $p < 0.01$

Fig. 16. Negative feedback regulation of Th1/Th2 cytokine balance mediated by DC and NKT cell interaction. This regulation system is important in bridging innate and acquired immunity.

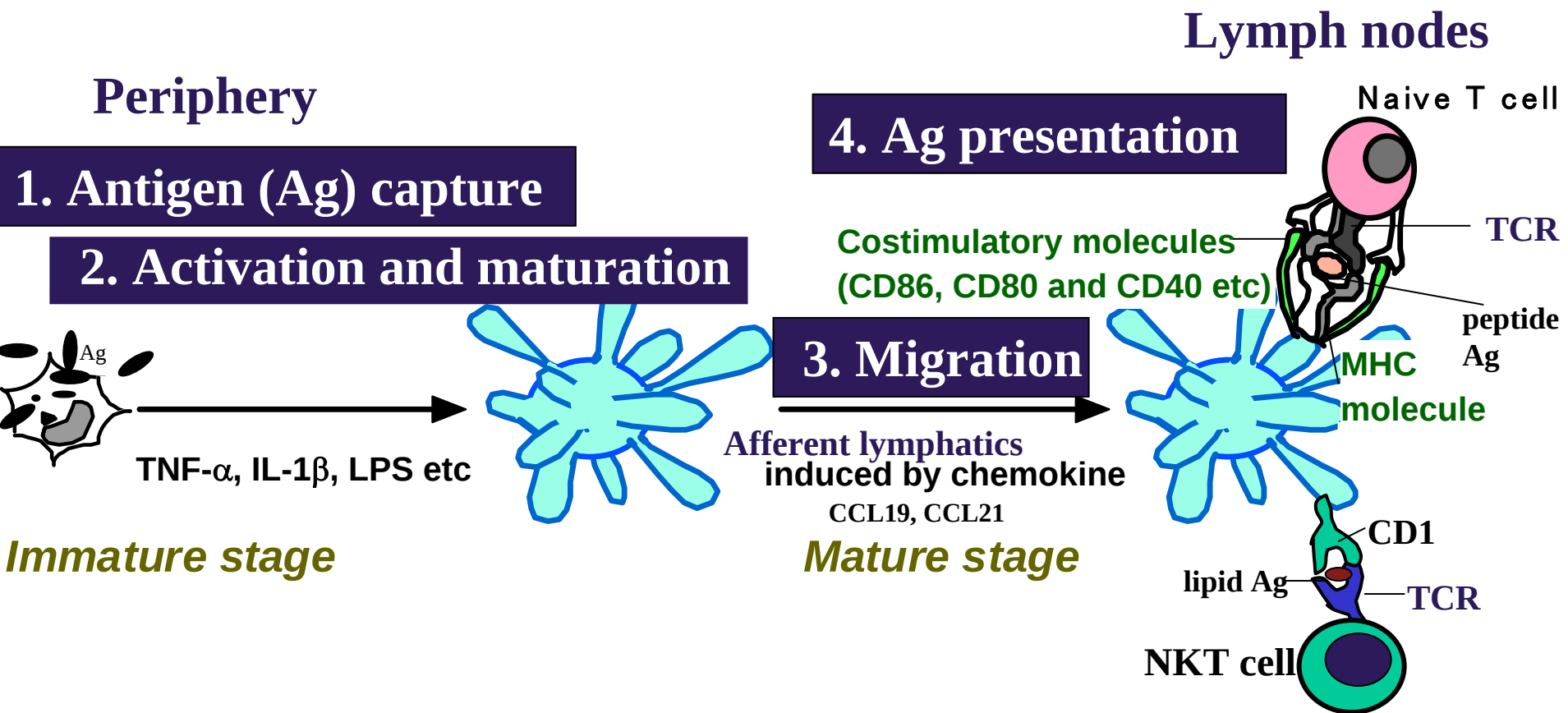
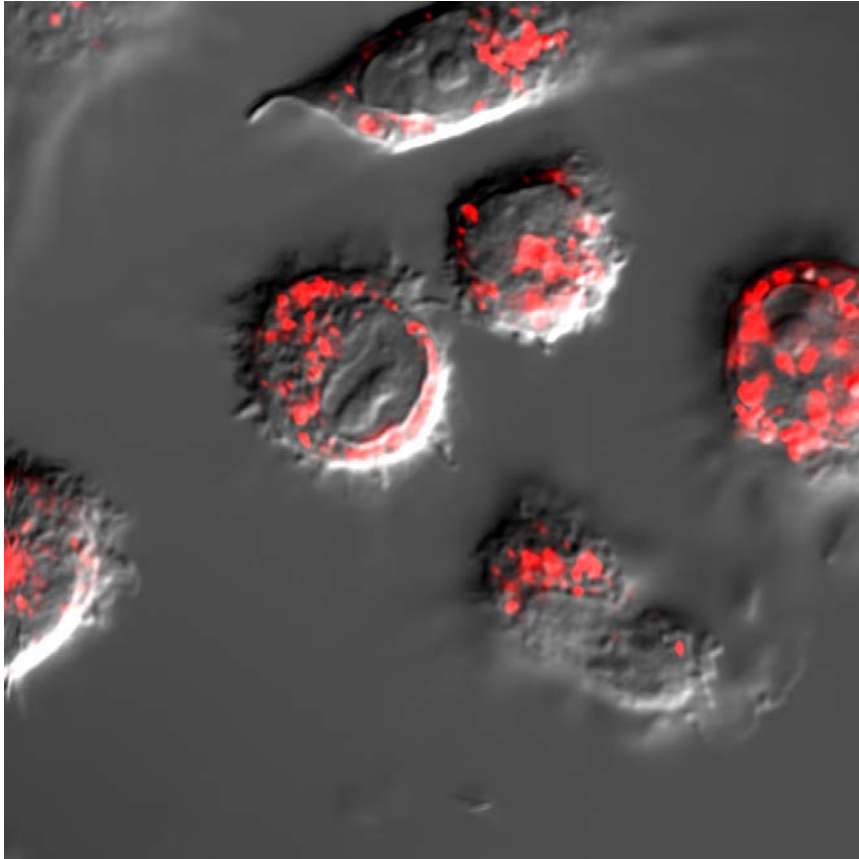


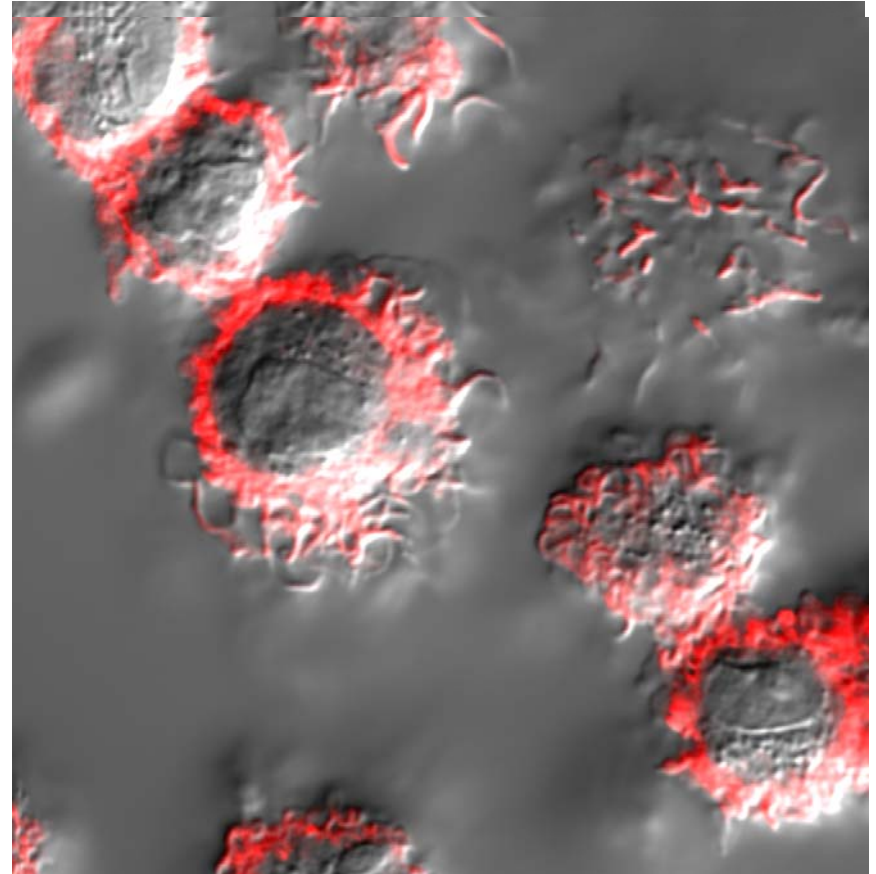
Fig. 1

Immature DCs



MHC class II

Mature DCs (treated with LPS)



MHC class II

Fig. 2

Dendritic cell

T cell

Adhesion
Molecules

DC-SIGN

CD54
(ICAM-1)

ICAM-3

LFA-1

MHC

TCR/CD3

T cell
activation

CD80

CD86

CD28

maturation

CD40

CD40 ligand (CD40L)

IL-12

Th1
shift

stimuli

Fig. 3

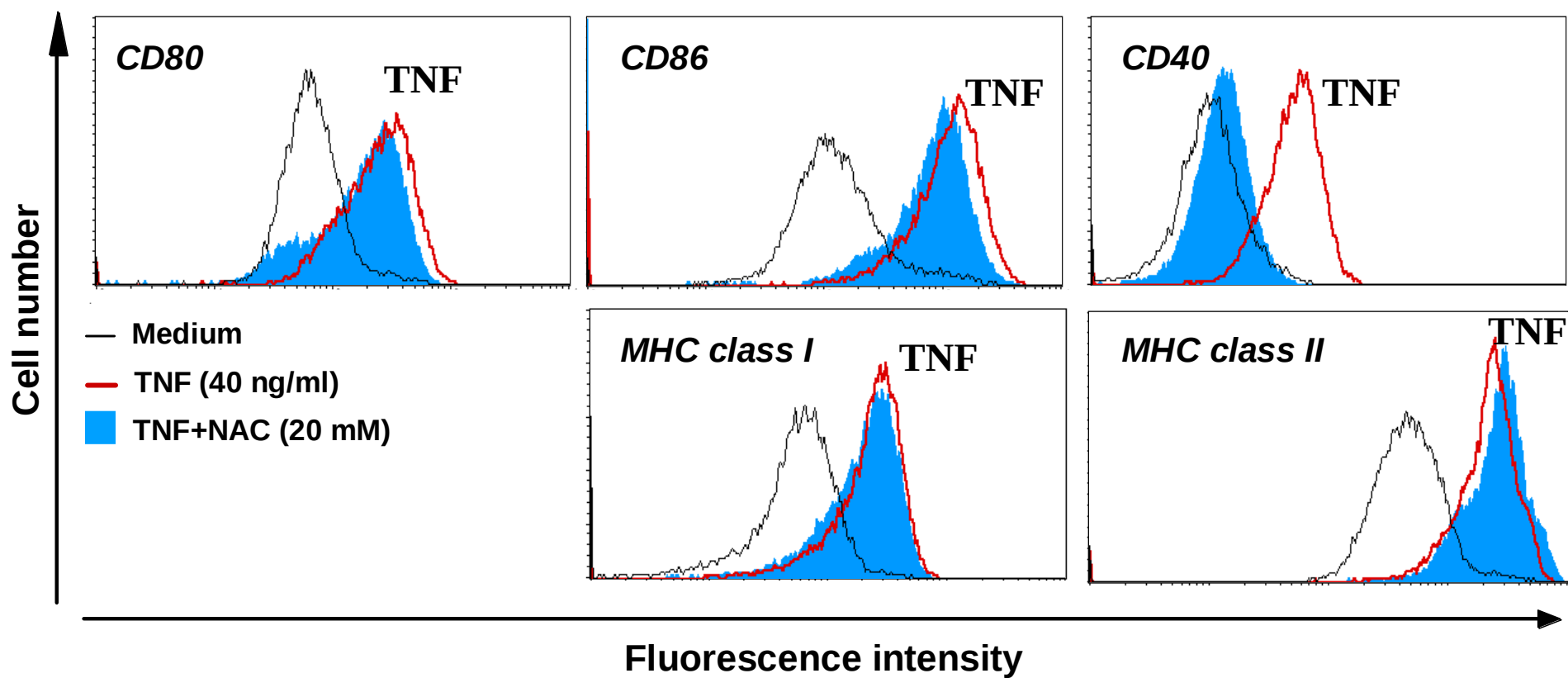


Fig. 4

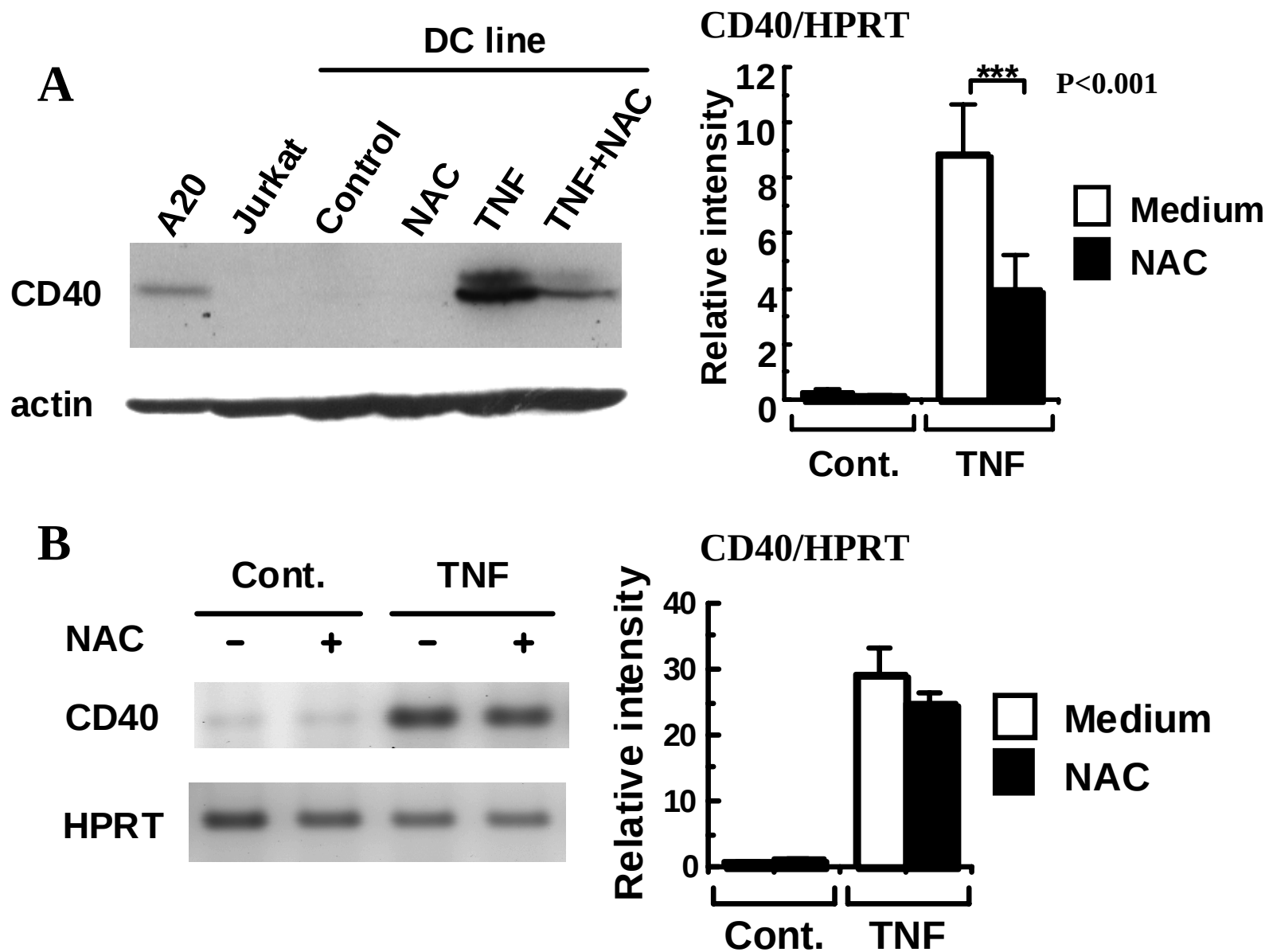


Fig. 5

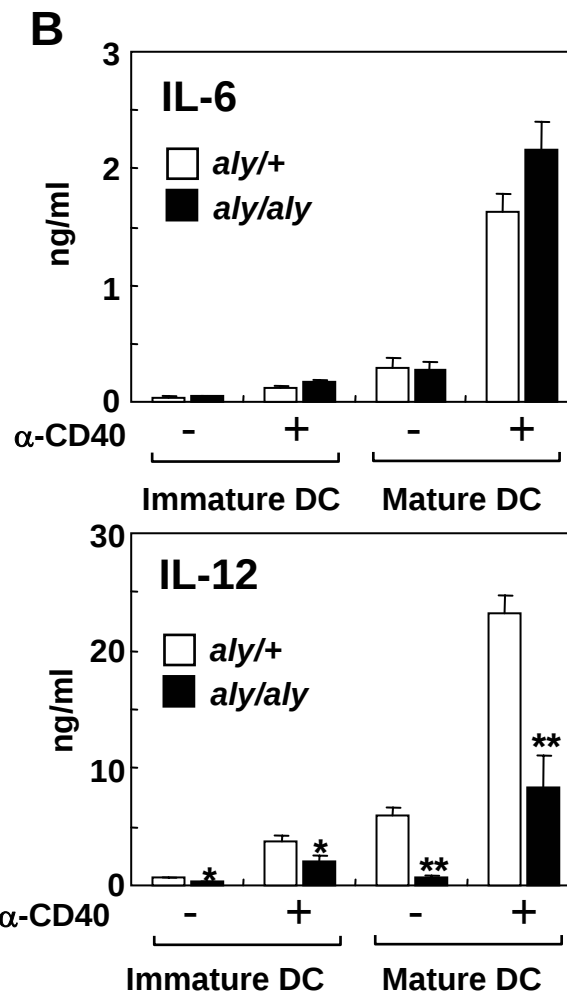
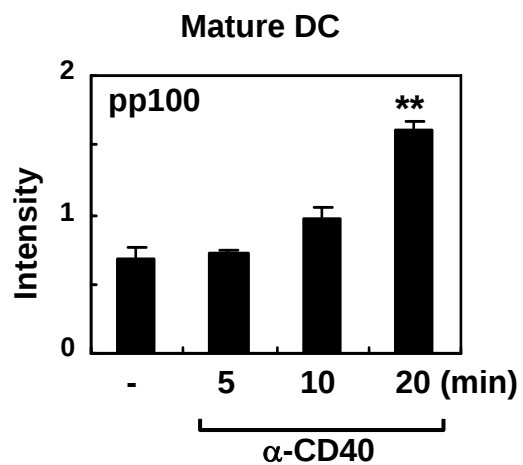
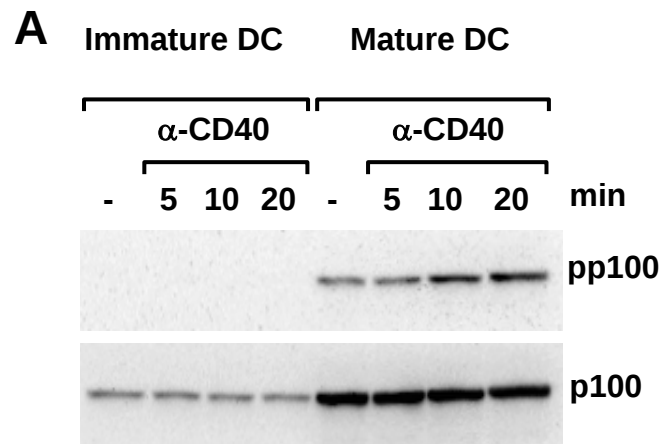


Fig. 6

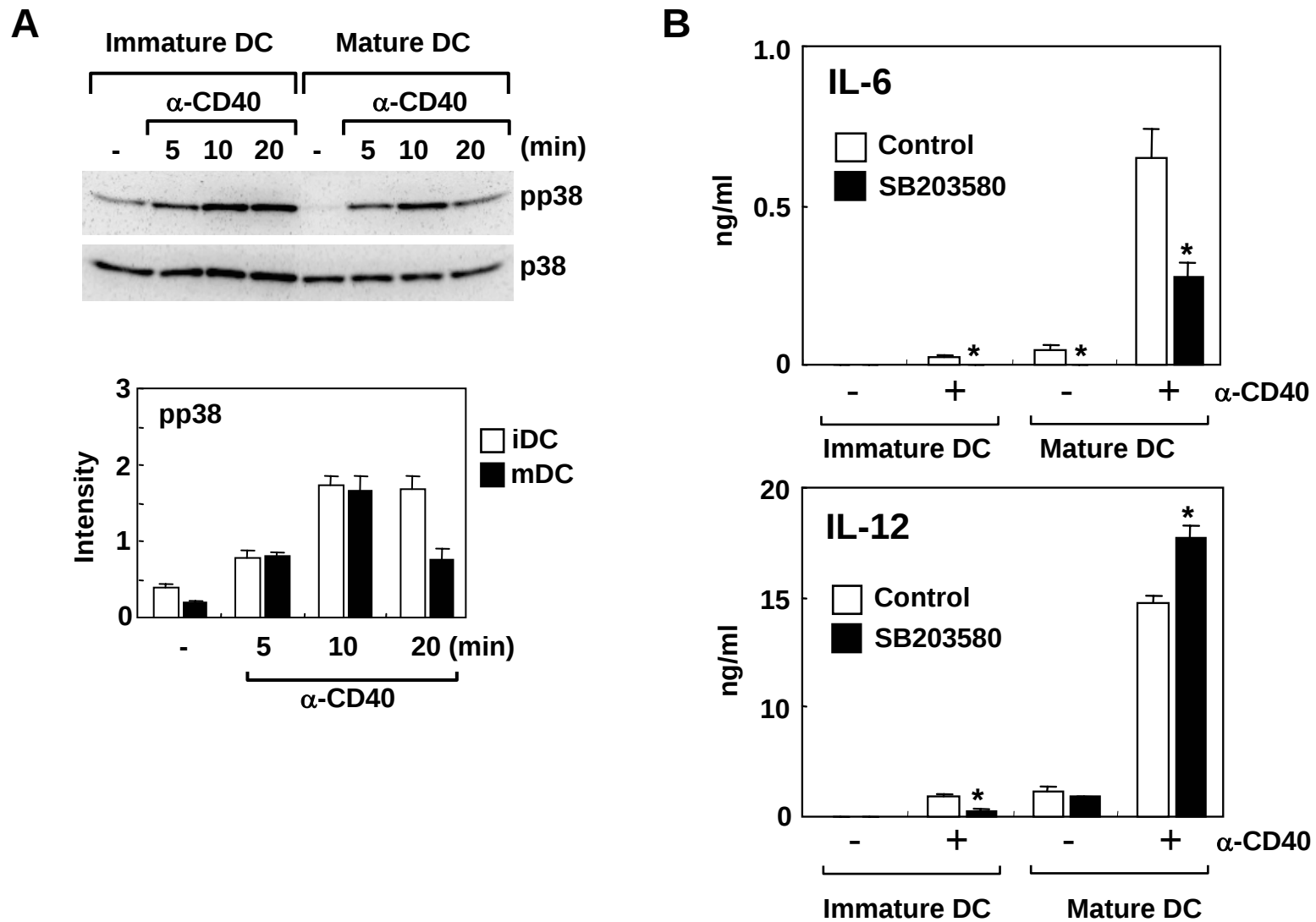


Fig. 7

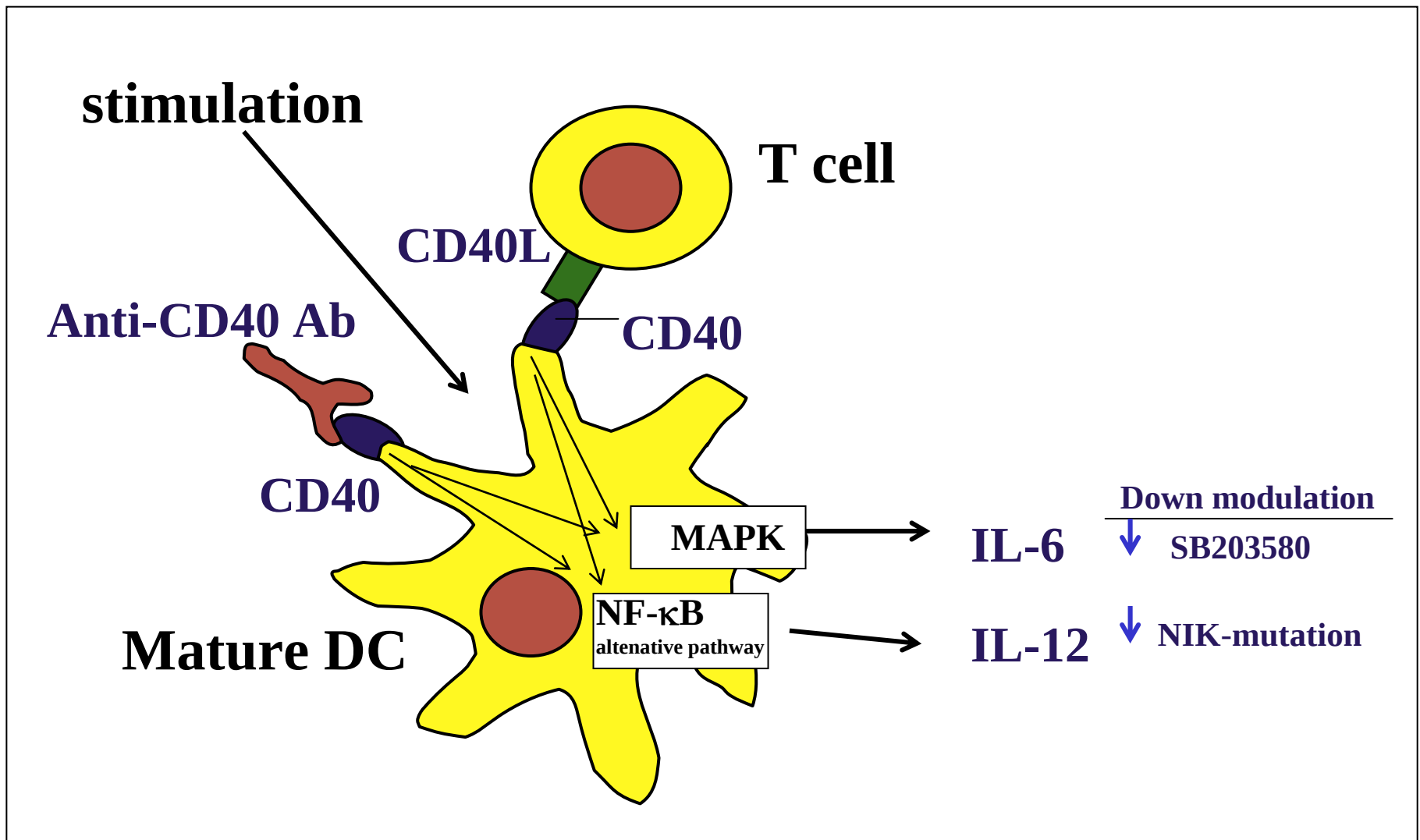


Fig. 8

Characteristics of NKT cells

1. Express both NK receptors (i.e. NK1.1) and limited TCR (i.e. V α 14, V β 8 in mouse and V α 24, V β 11 in human) (Ref. 12, 21, 26,)
2. Quickly produce a large amount of various cytokines such as IFN- γ , IL-4, osteopontin and IL-13 upon stimulation (Ref. 2, 7, 22, 23)
3. Kill tumor cells and CD4⁺8⁺ thymocytes expressing Fas (Ref. 24, 25)
4. Respond to oxidized lipid antigens, produce IFN- γ and aggravates atherosclerosis (Ref. 3)

atherosclerosis

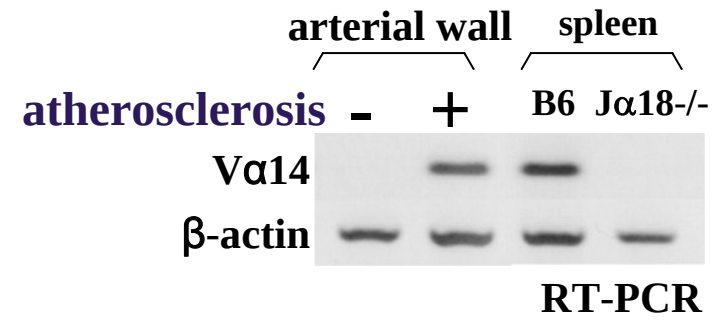


Fig. 9

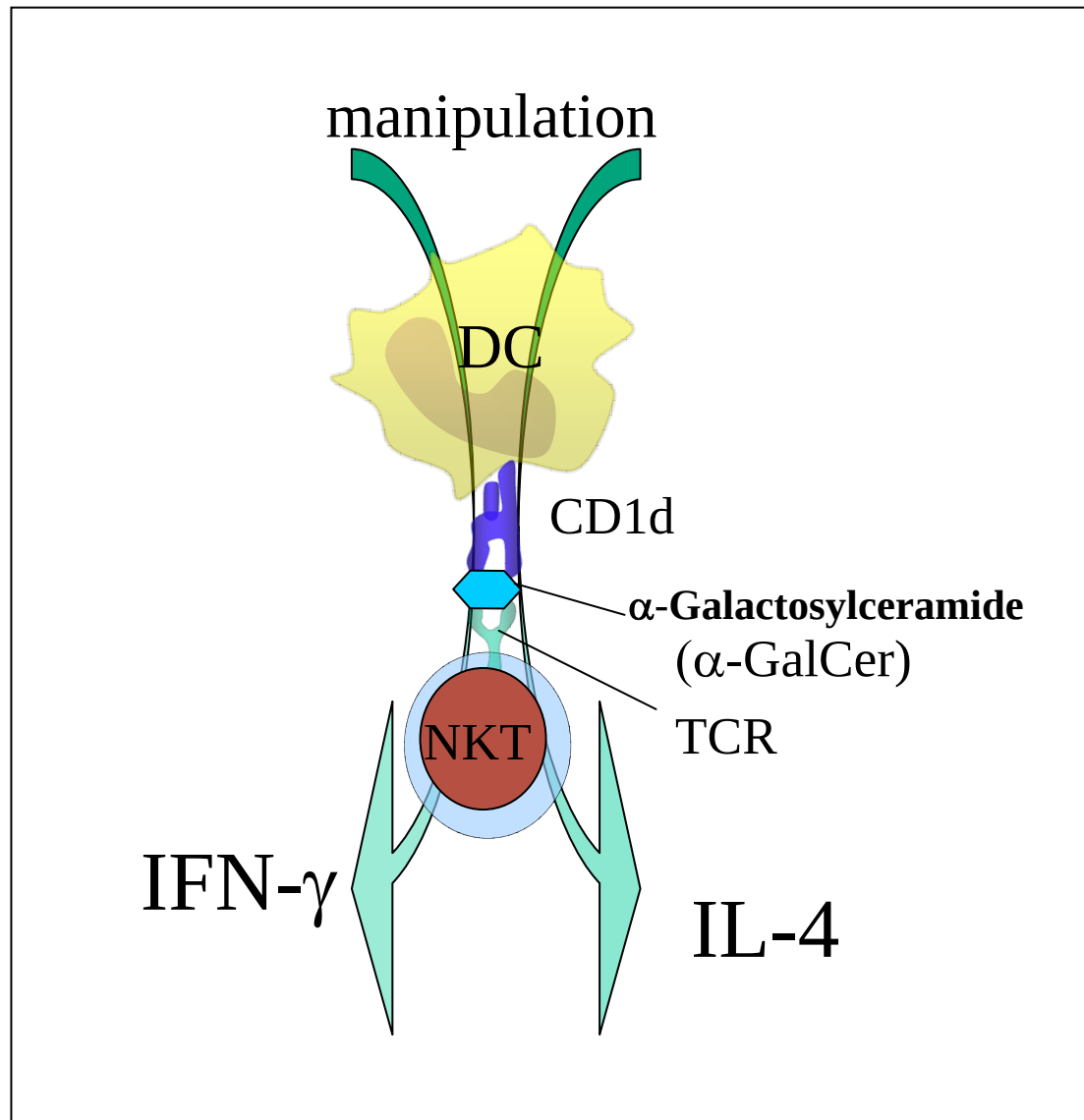
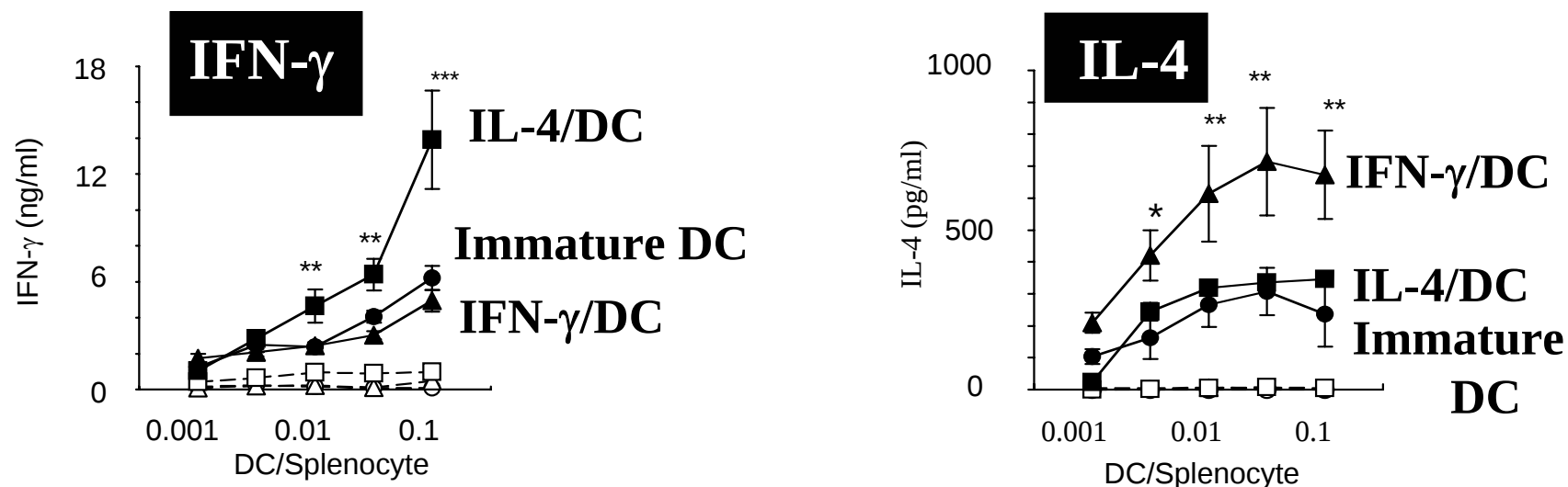
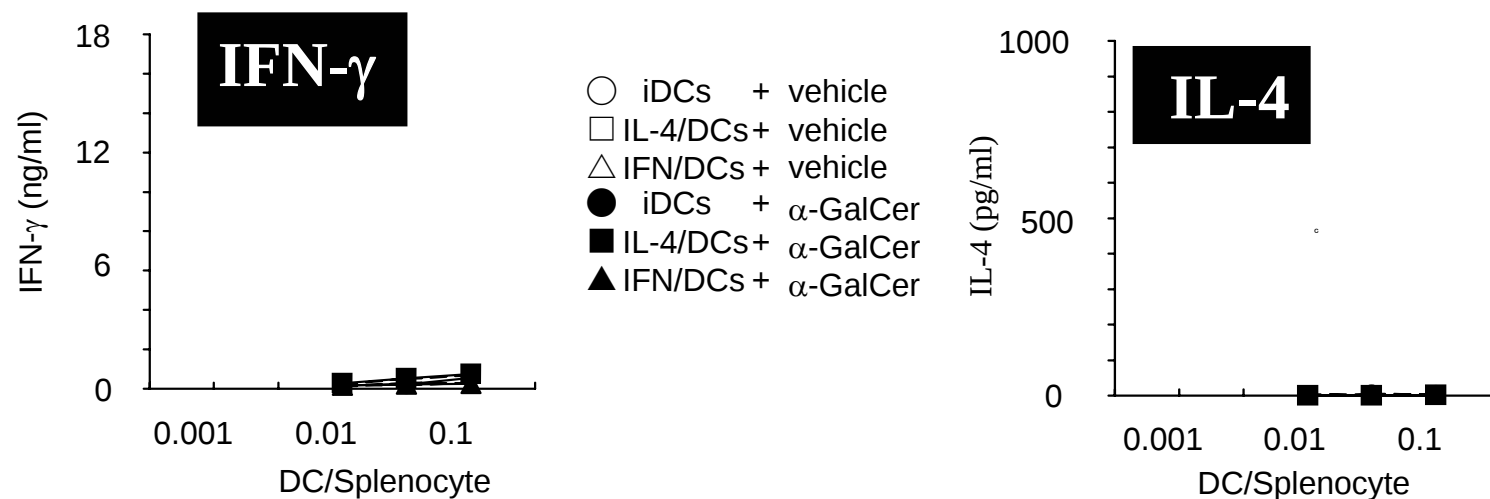


Fig. 10

A**DCs vs. BALB/c splenocytes****B****DCs vs. CD1d KO splenocytes****Fig. 11**

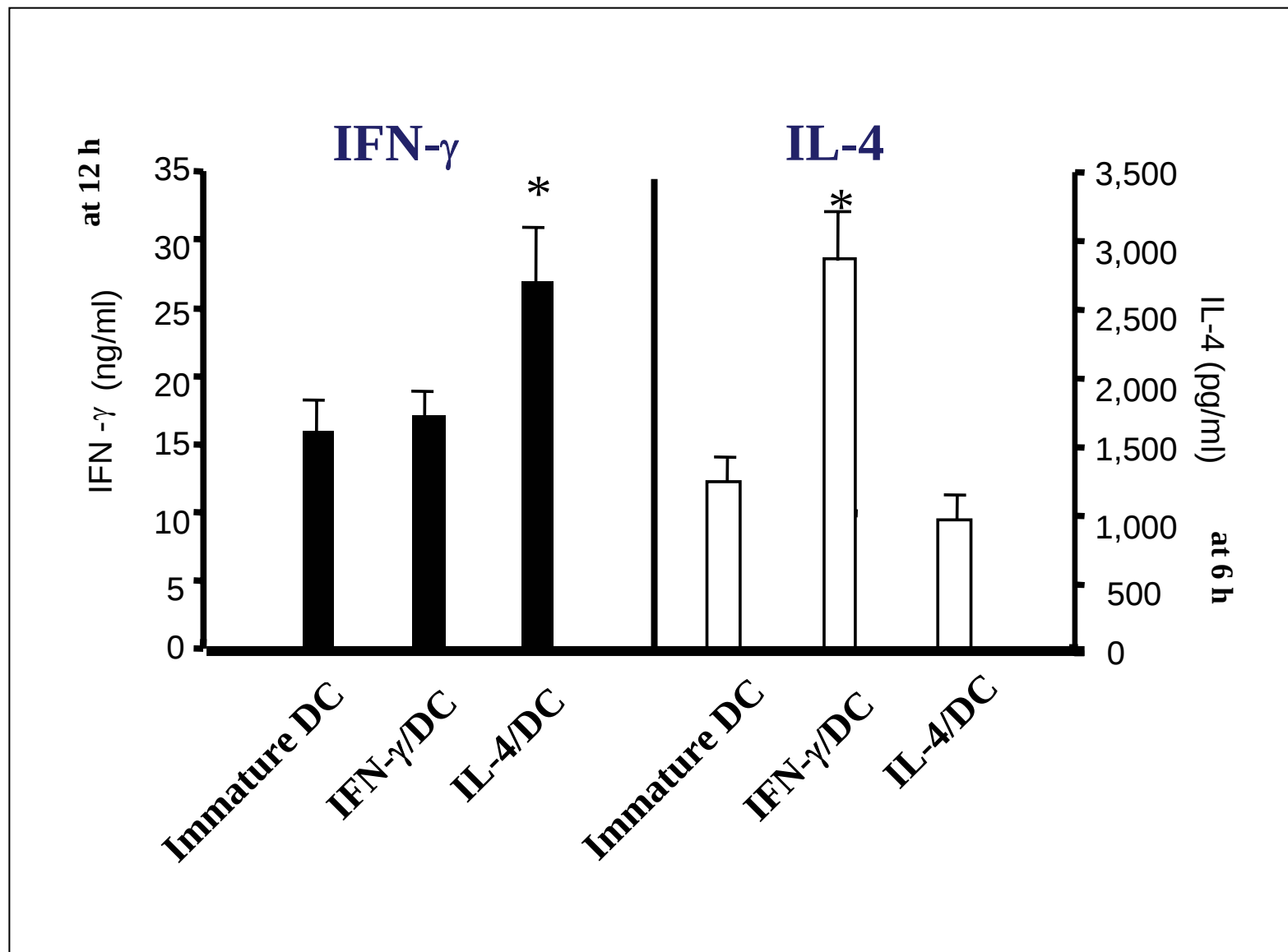


Fig. 12

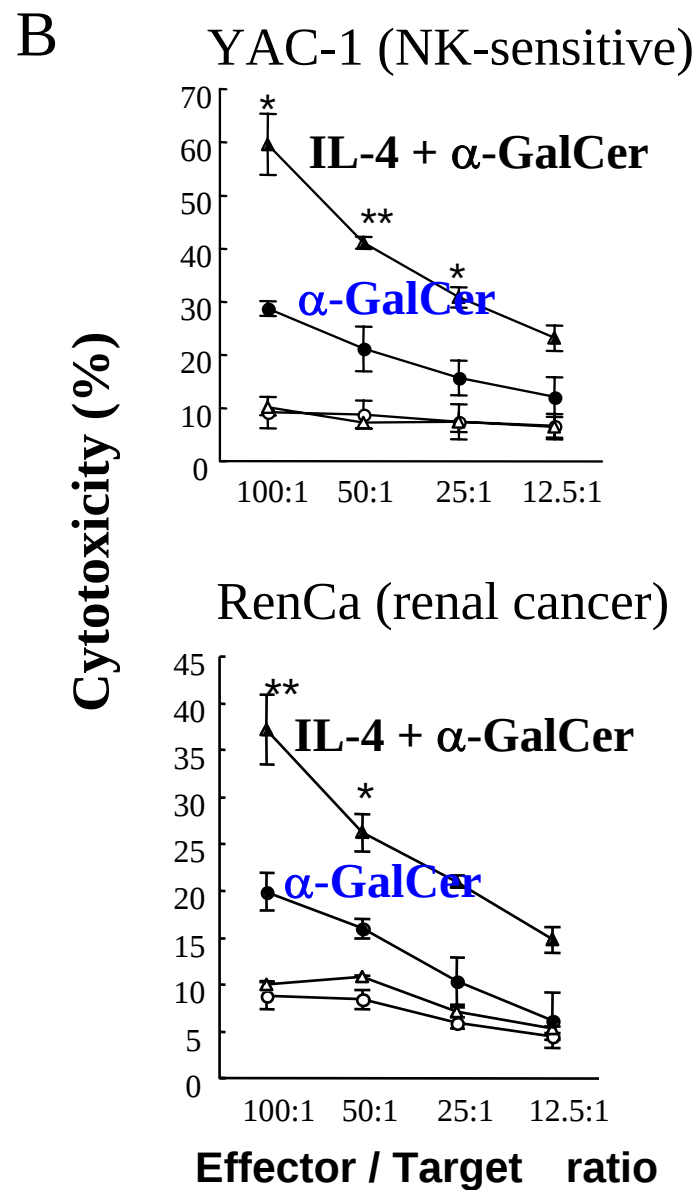
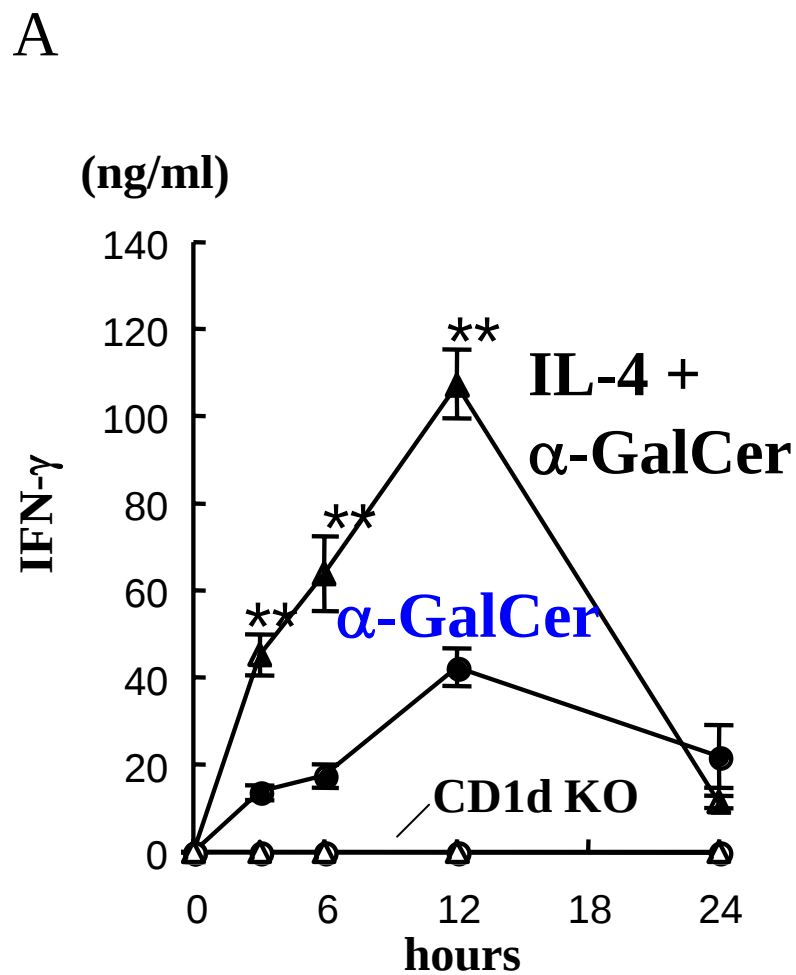


Fig. 13

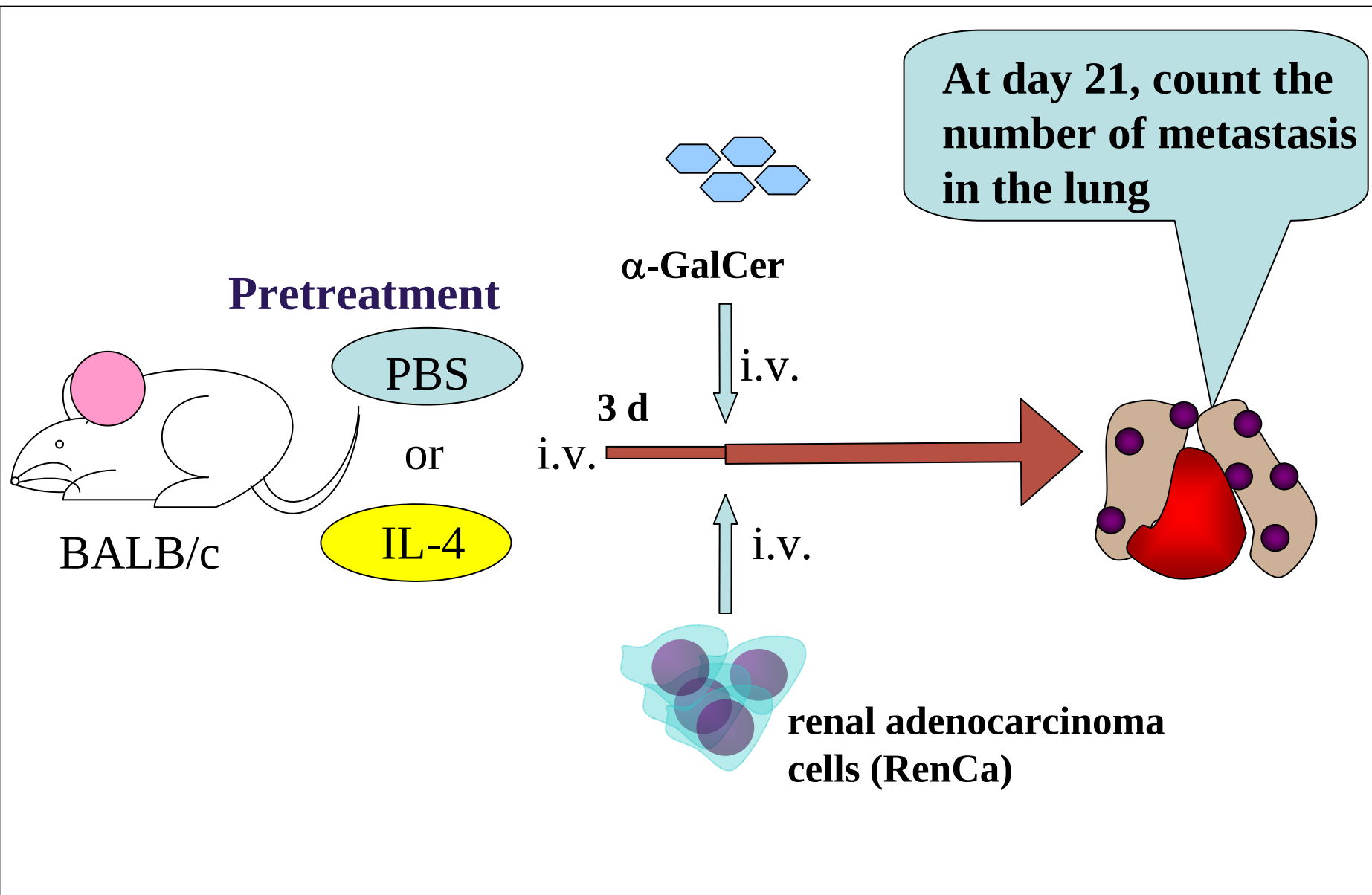
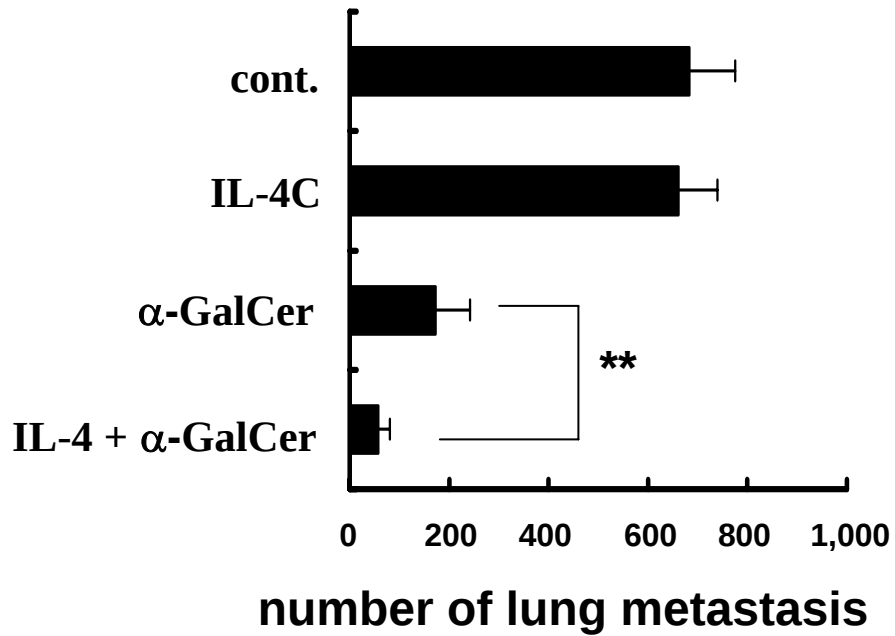


Fig. 14

A



B

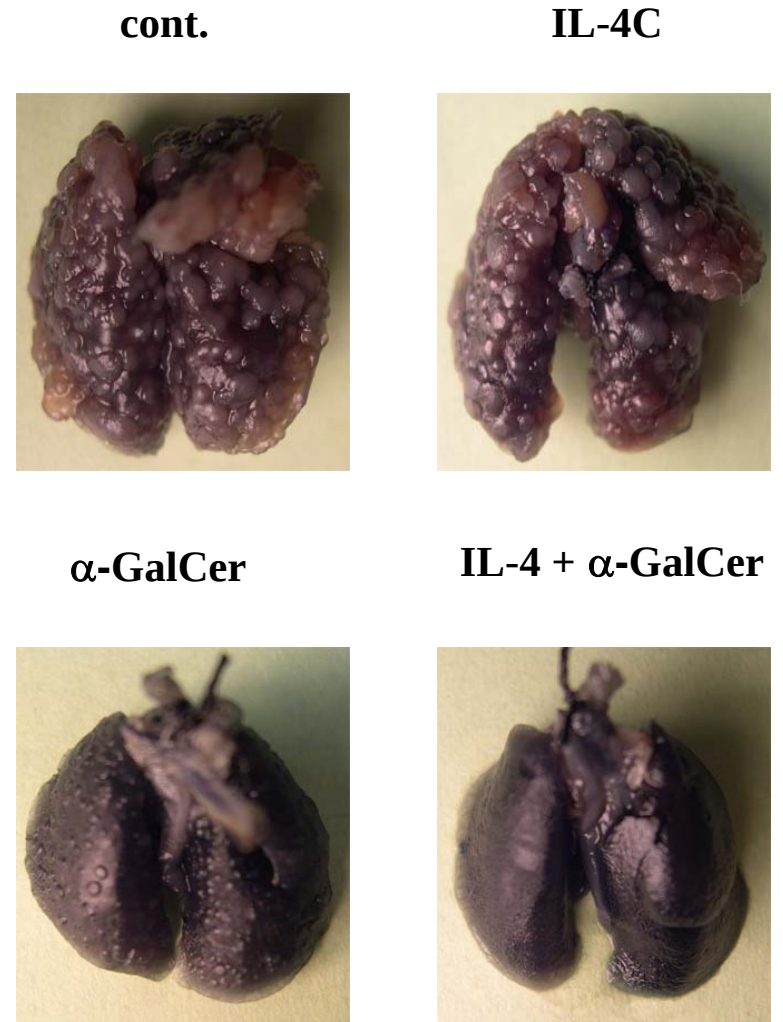


Fig. 15

1. NKT-produced Th1/Th2 cytokine balance is under control of negative feedback loop via DC. Th1/DCs induced Th2 cytokine and Th2/DCs induced Th1 cytokine in NKT cells.
2. IL-4 plus α -GalCer induced vigorous anti-cancer reaction in vivo. The negative feedback regulation system could be applicable to therapeutics of cancer, various infectious diseases and autoimmune diseases.

Negative feedback regulation

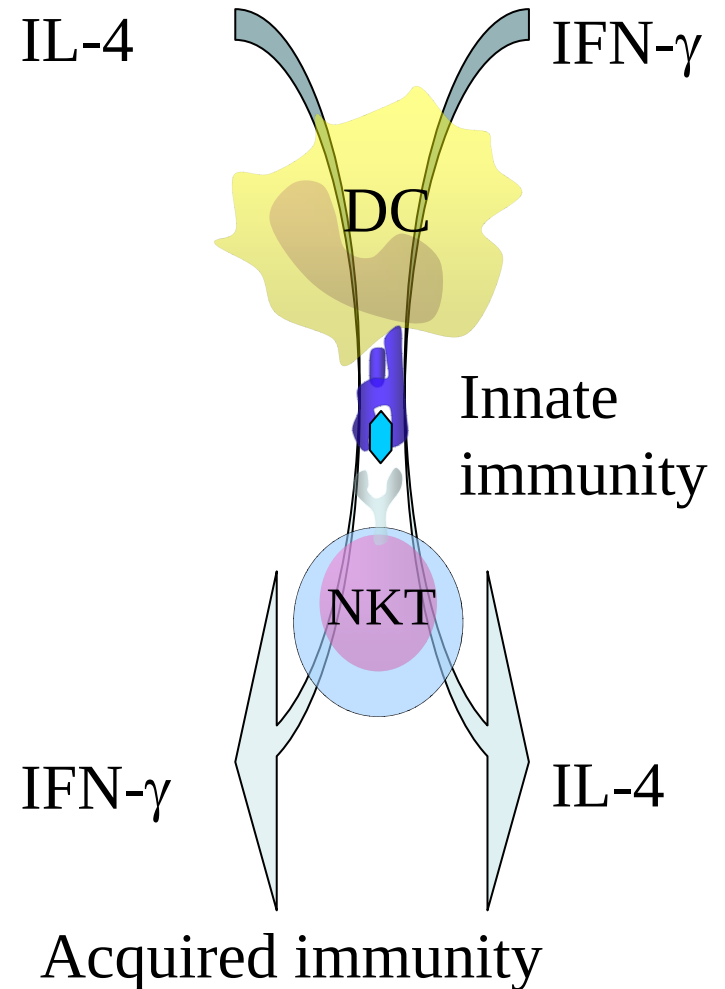


Fig. 16