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

Kazunori Onoé, Yoshiki Yanagwa, Keita Minami, Norifumi Iijima, and Kazuya Iwabuchi

Division of Immunobiology

Institute for Genetic Medicine, Hokkaido University

Corresponding author: Kazunori Onoé, M.D., Ph.D. Division of Immunobiology

Institute for Genetic Medicine, Hokkaido University, Kita-ku, Kita-15, Nishi-7,

Sapporo, 060-0815, Japan

Tel: 011-706-5531 FAX: 011-706-7545

E-mail: kazunori@igm.hokudai.ac.jp

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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 athrosclerosis *(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 Costuimulatory 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 maturate. Mature DCs then migrate to lymph nodes in the presence of chemokines, CCL19 and CCL21. These mature DCs express high levels of major

histocompatibility comples (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 pthways *(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 NK-κB 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 NK-κB 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-κB inducing kinase *(20).* Thus, these findings suggest that NF- κB 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 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-κB 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-κB 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 Vα24 and Vβ11 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 Vα chain, Vα14Jα18, paired with a restricted Vβ 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 Vα14Jα18. 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 Vα14 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 immnunosorbent 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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Table 1 Two Aspects of Th1 and Th2 Responses

Th1 response (cellular)	1. Mediated by IFN-γ, IL-2, TNF-α 2. Generates protective responses against viruses, bacteria and tumor cells 3. Aggravates certain types of autoimmune diseases and atherosclerosis
Th2 response (humoral)	1. Mediated by IL-4, IL-10, IL-13 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-κB 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-κB activation and cytokine production following stimulation through CD40. DCs derived from *aly/+* and *aly/aly* NF-κB inducing kinase (NIK) mutant mice were analyzed (Ref. 19). A. NF-κB is activated by anti-CD40 mAb in mature DCs. B. IL-6 and IL-12 production by DCs stimulated through CD40. NF-κB 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-κB 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 Vα 14 Jα 18 mRNA was analyzed by RT-PCR (right bottom panel) (Ref. 3). Note the prominent expression of Vα14 mRNA in the atherosclerotic lesion, whereas no expression of the Vα14 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 intraveneously injected with RenCa cells (5×10^5) and α-GalCer (2μg/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 ± 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.

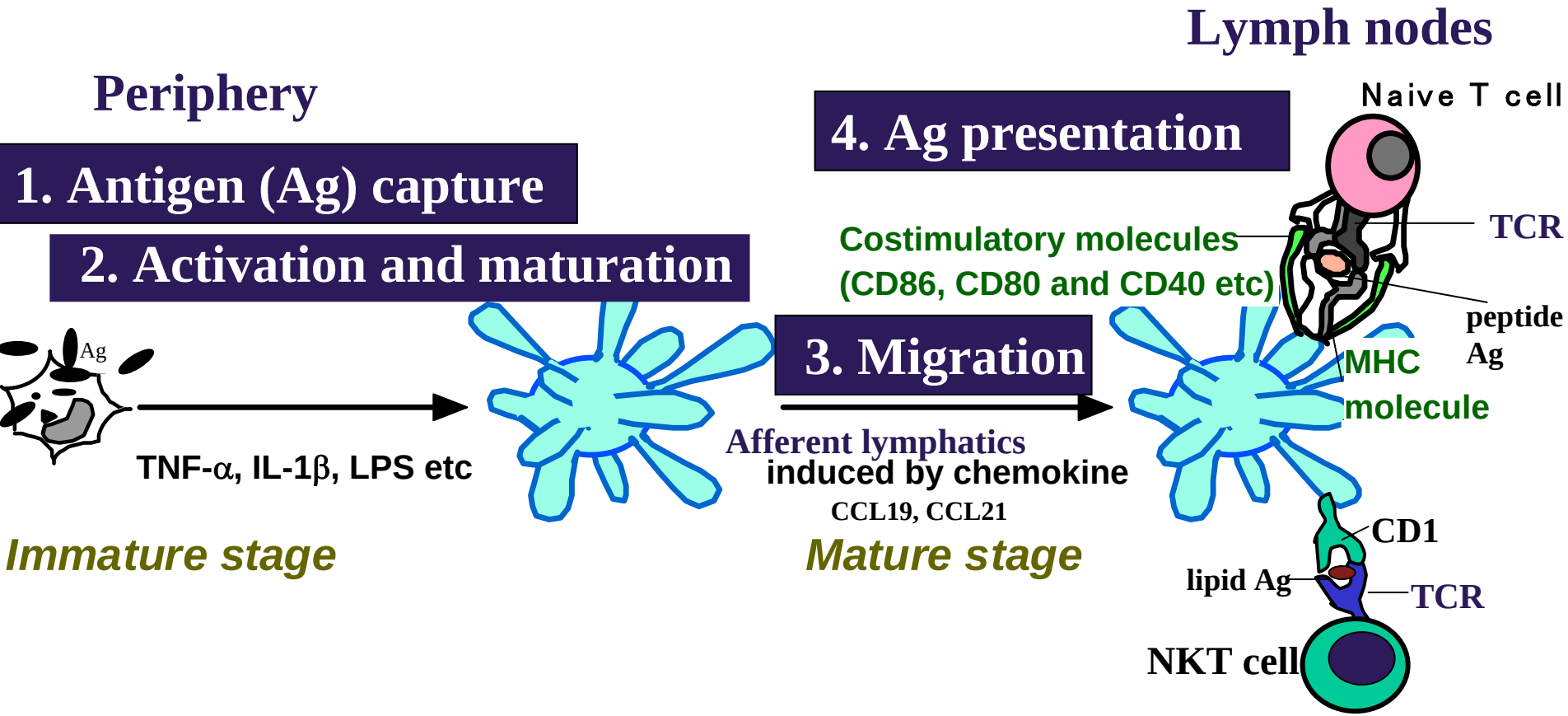
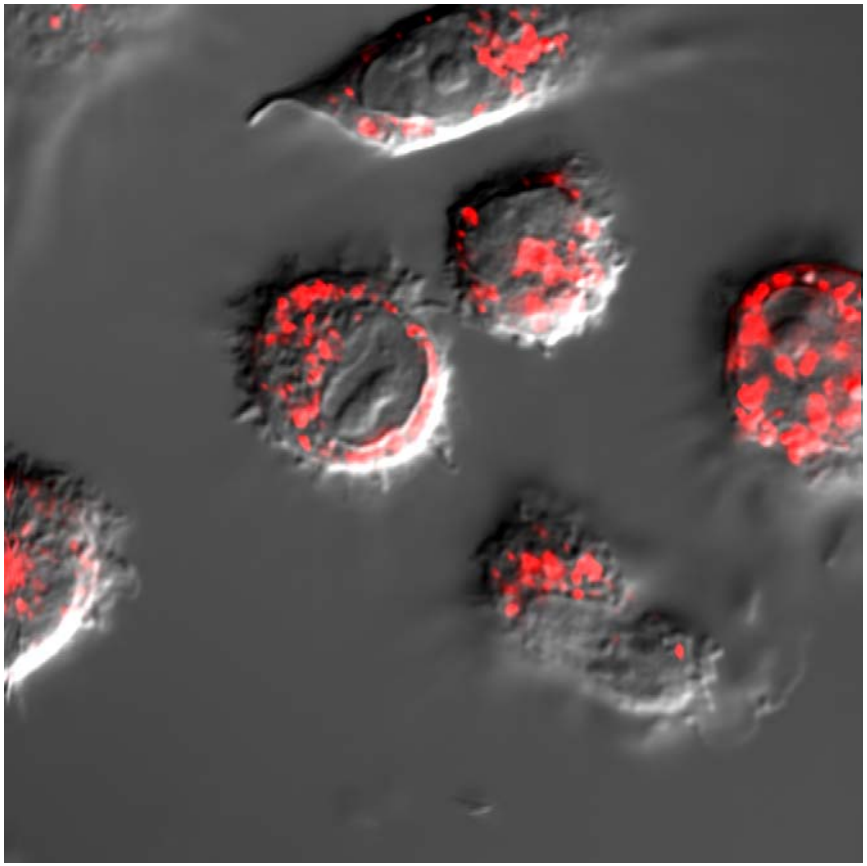

Lymph nodes
Periphery
4. Ag presentation
Naive T cell
1. Antigen (Ag) capture
TCR
Costimulatory molecules
(CD86, CD80 and CD40 etc)
2. Activation and maturation
peptide
Ag
Ag
3. Migration
MHC
molecule
Afferent lymphatics
induced by chemokine
CCL19, CCL21
TNF-α, IL-1β, LPS etc
CD1
lipid Ag
TCR
NKT cell
Immature stage
Mature stage

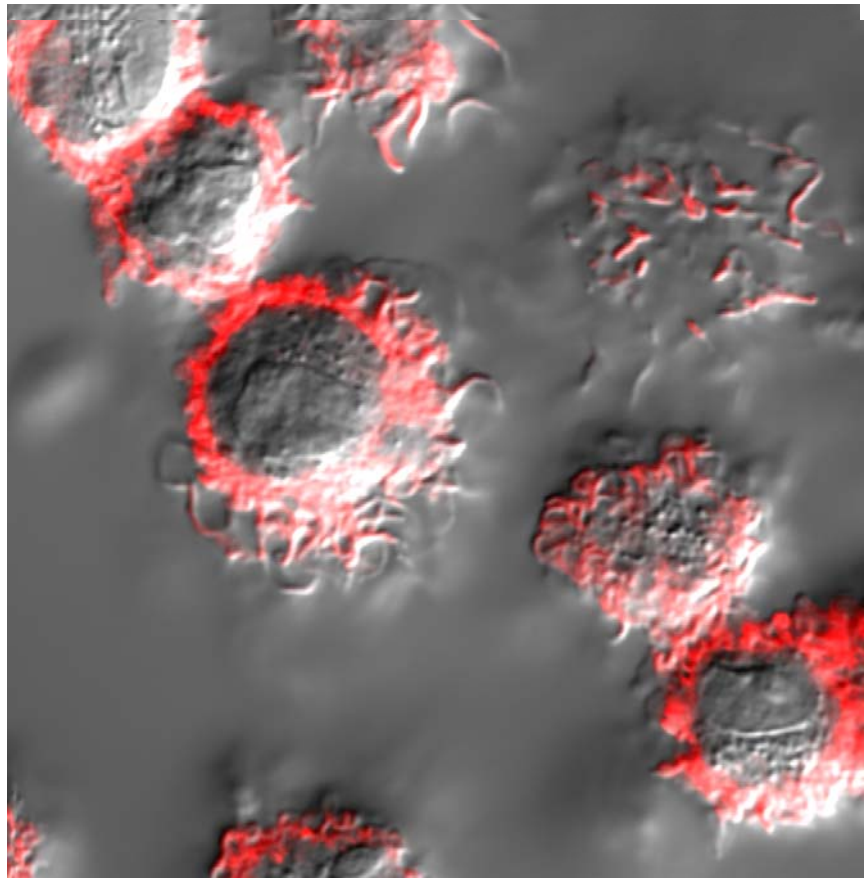
Fig. 1

Immature DCs



MHC class II

Mature DCs (treated with LPS)



MHC class II

Fig. 2

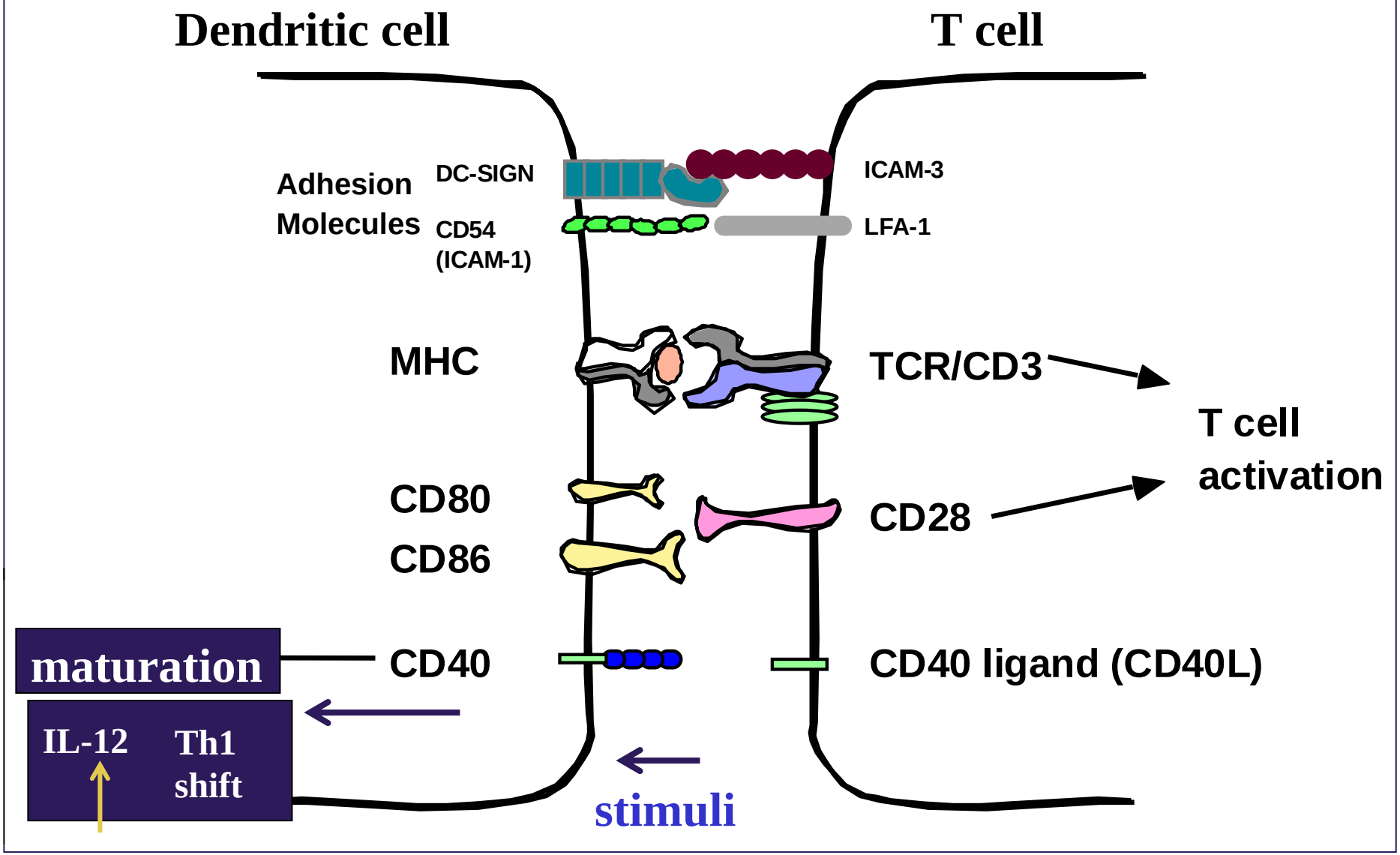

Dendritic cell
T cell
Adhesion Molecules
DC-SIGN
CD54 (ICAM-1)
ICAM-3
LFA-1
MHC
TCR/CD3
T cell activation
CD80
CD86
CD28
maturation
CD40
CD40 ligand (CD40L)
IL-12
Th1 shift
stimuli

Fig. 3

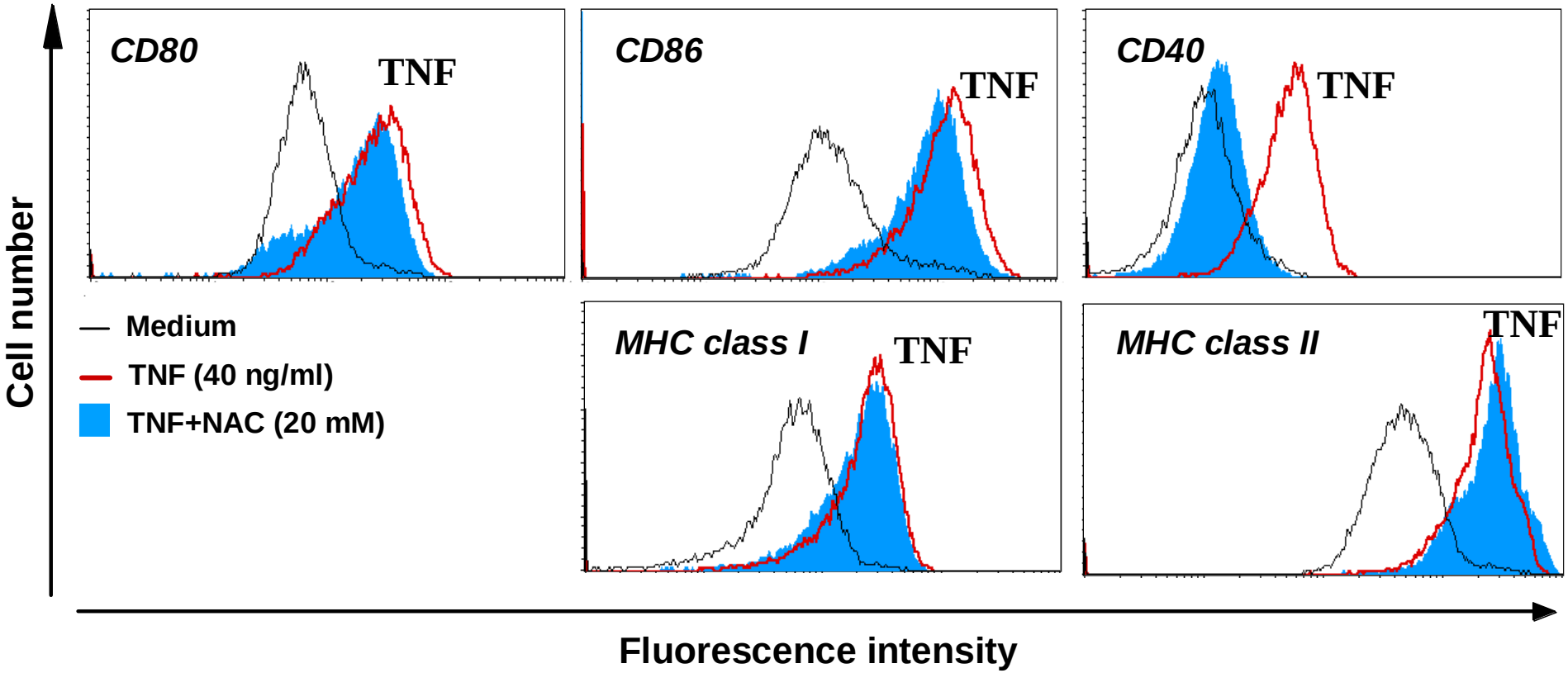

CD80
TNF
CD86
TNF
CD40
TNF
MHC class I
TNF
MHC class II
TNF
Medium
TNF (40 ng/ml)
TNF+NAC (20 mM)
Cell number
Fluorescence intensity

Fig. 4

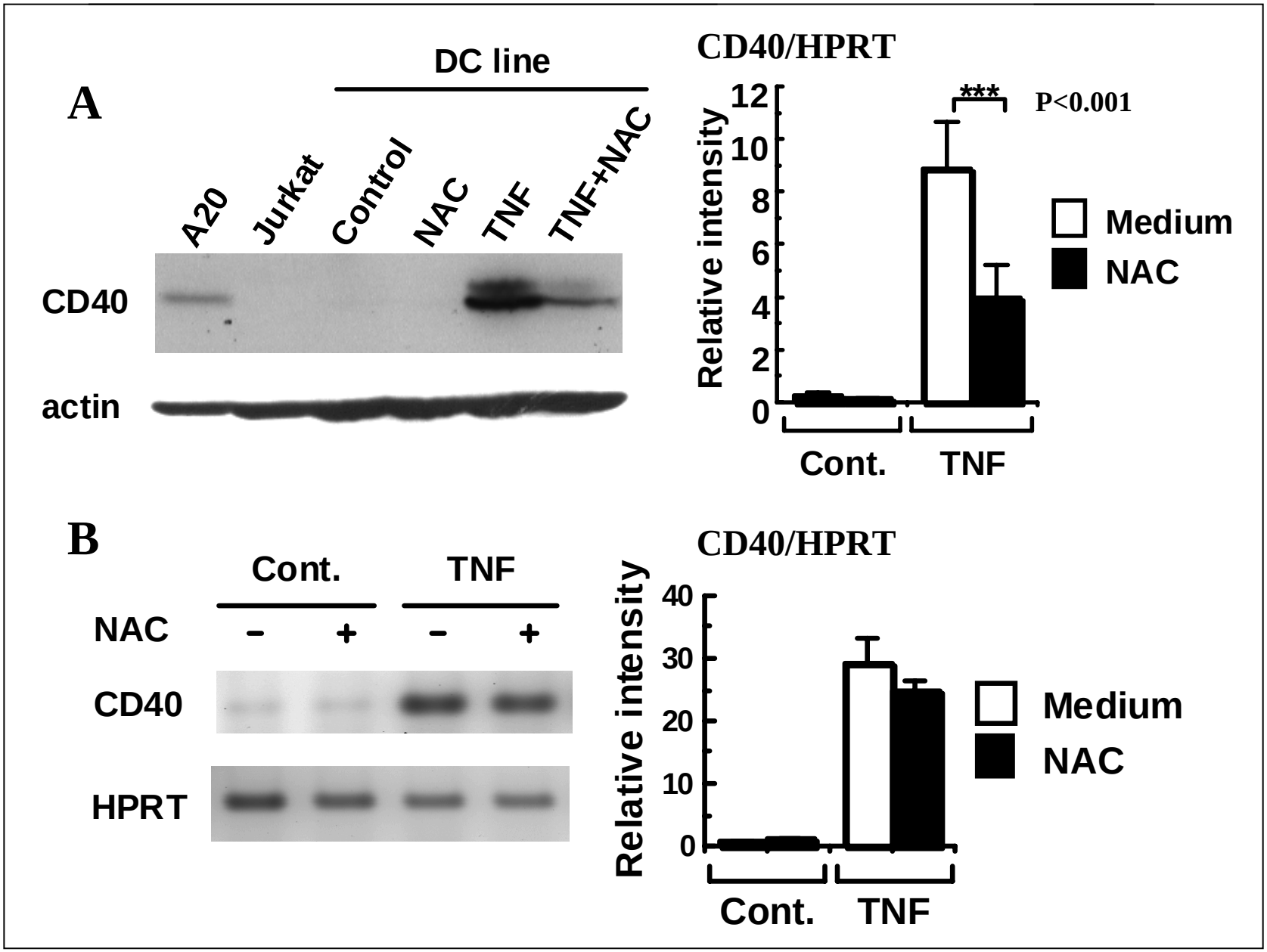
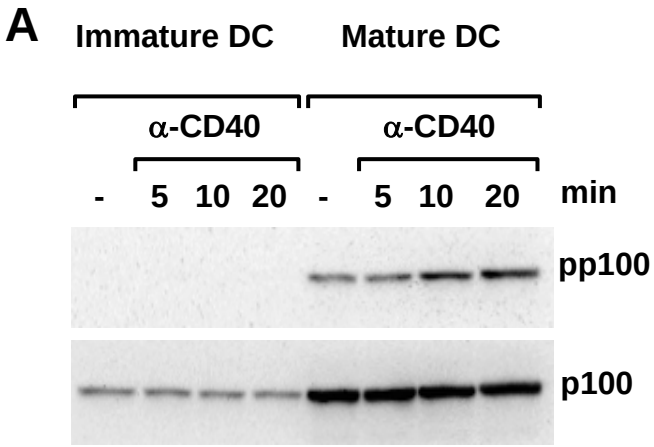
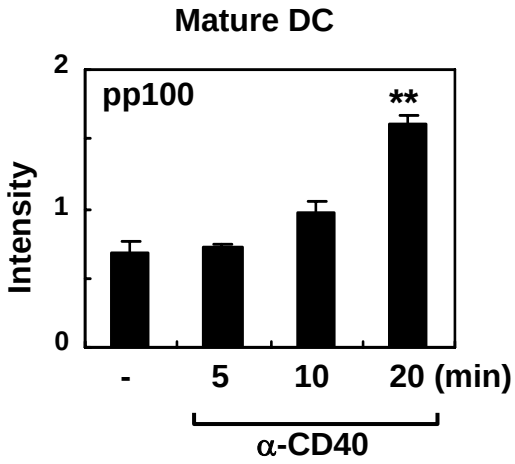
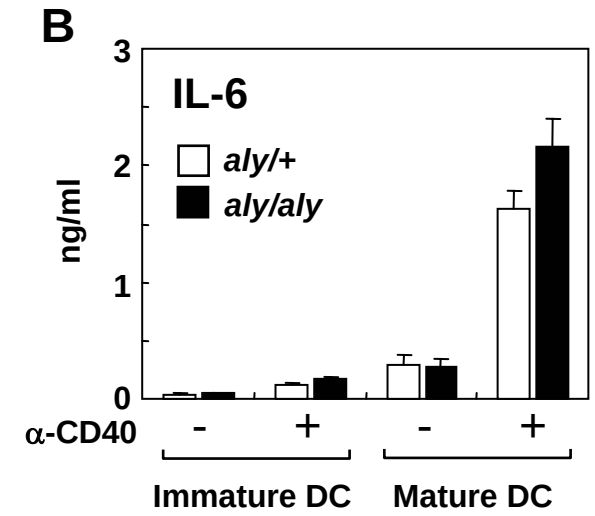

A
DC line
A20
Jurkat
Control
NAC
TNF
TNF+NAC
CD40
actin
CD40/HPRT
***
P<0.001
Relative intensity
12
10
8
6
4
2
0
Medium
NAC
Cont.
TNF
B
Cont.
TNF
NAC
–
+
–
+
CD40
HPRT
CD40/HPRT
Relative intensity
40
30
20
10
0
Medium
NAC
Cont.
TNF

Fig. 5


A
Immature DC
Mature DC
α-CD40
α-CD40
- 5 10 20 - 5 10 20
min
pp100
p100


Mature DC
pp100
**
Intensity
2
1
0
- 5 10 20 (min)
α-CD40


B
IL-6
aly/+
aly/aly
ng/ml
3
2
1
0
α-CD40
- + - +
Immature DC
Mature DC

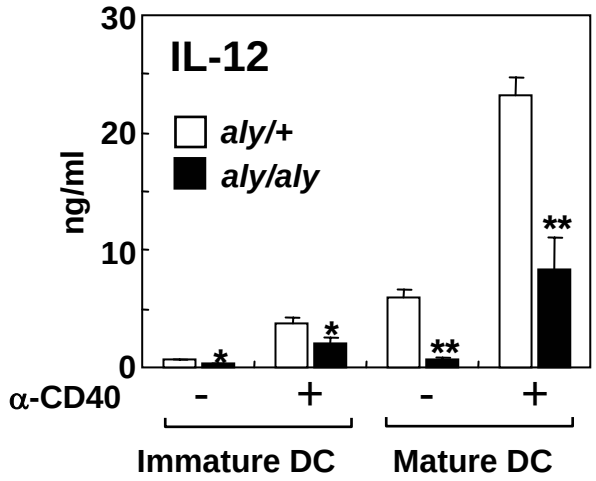

IL-12
aly/+
aly/aly
ng/ml
30
20
10
0
*
*
**
**
α-CD40
- + - +
Immature DC
Mature DC

Fig. 6

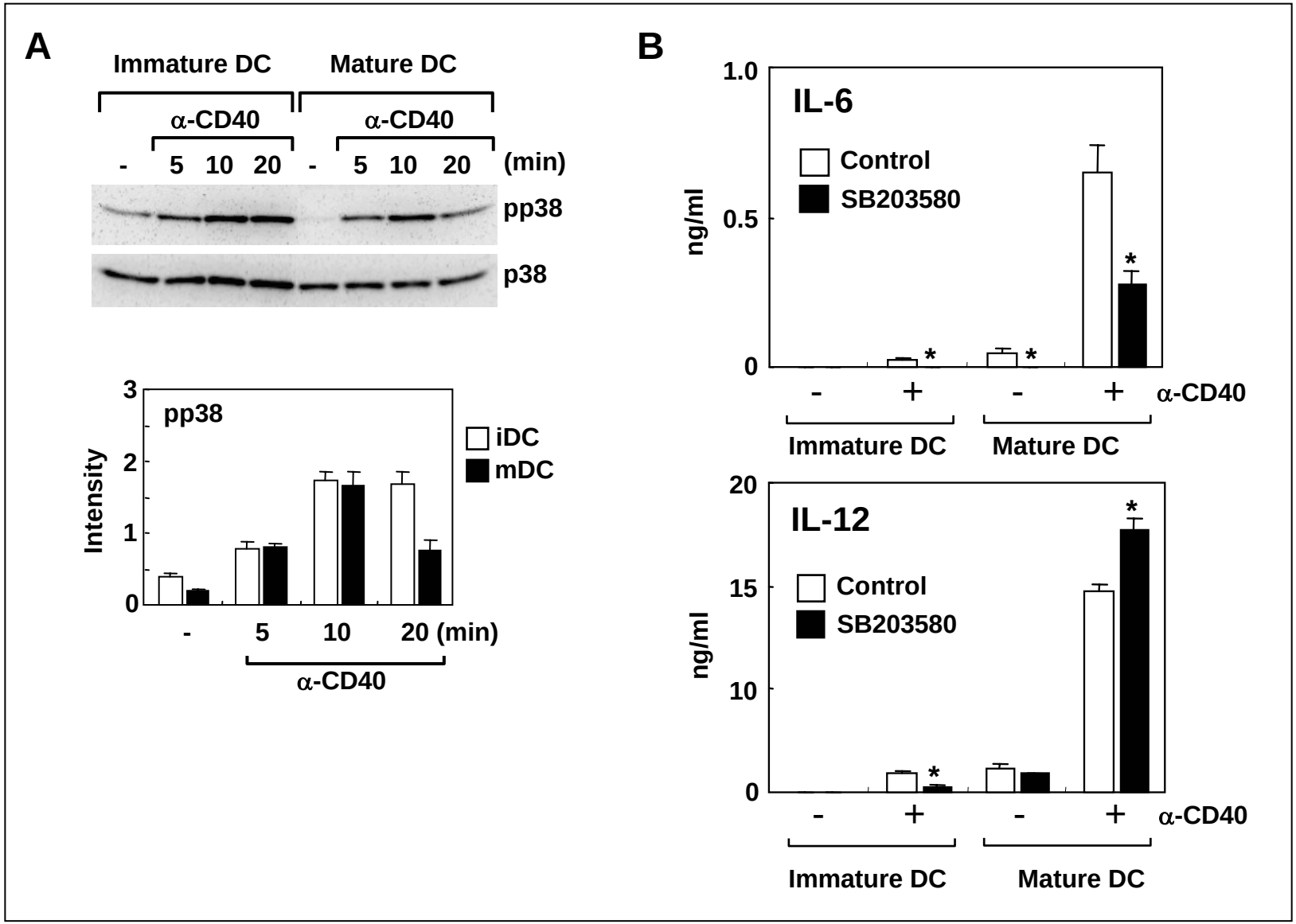

A
Immature DC
Mature DC
α-CD40
α-CD40
- 5 10 20 - 5 10 20 (min)
pp38
p38
pp38
Intensity
iDC
mDC
- 5 10 20 (min)
α-CD40
B
IL-6
Control
SB203580
ng/ml
1.0
0.5
0
- + - + α-CD40
Immature DC
Mature DC
IL-12
Control
SB203580
ng/ml
20
15
10
0
- + - + α-CD40
Immature DC
Mature DC

Fig. 7

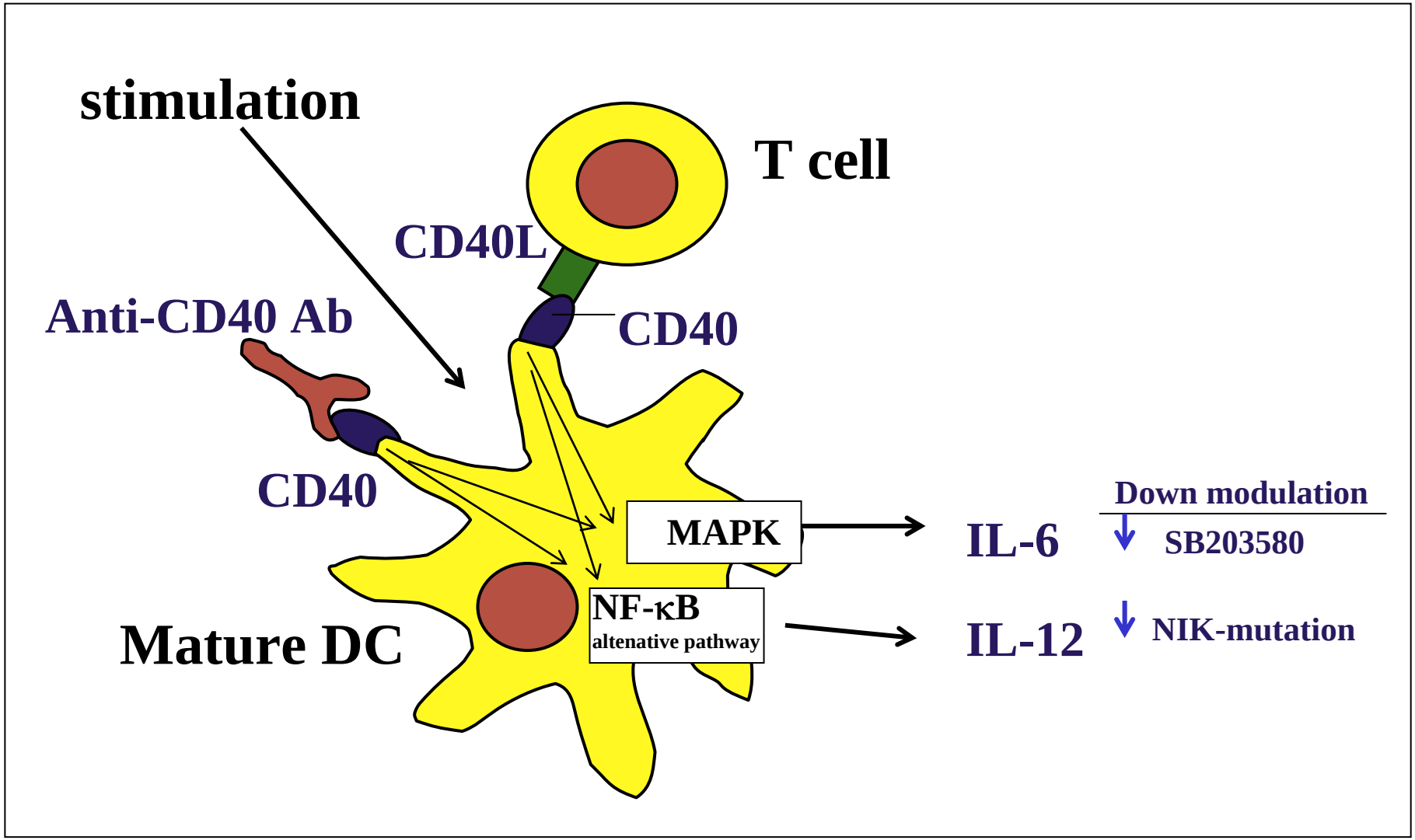

stimulation
T cell
CD40L
Anti-CD40 Ab
CD40
CD40
MAPK
NF-κB
altenative pathway
Mature DC
IL-6
IL-12
Down modulation
SB203580
NIK-mutation

Fig. 8

Characteristics of NKT cells

1. Express both NK receptors (i.e. NK1.1) and limited TCR (i.e. Vα14, Vβ8 in mouse and Vα24, Vβ11 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)

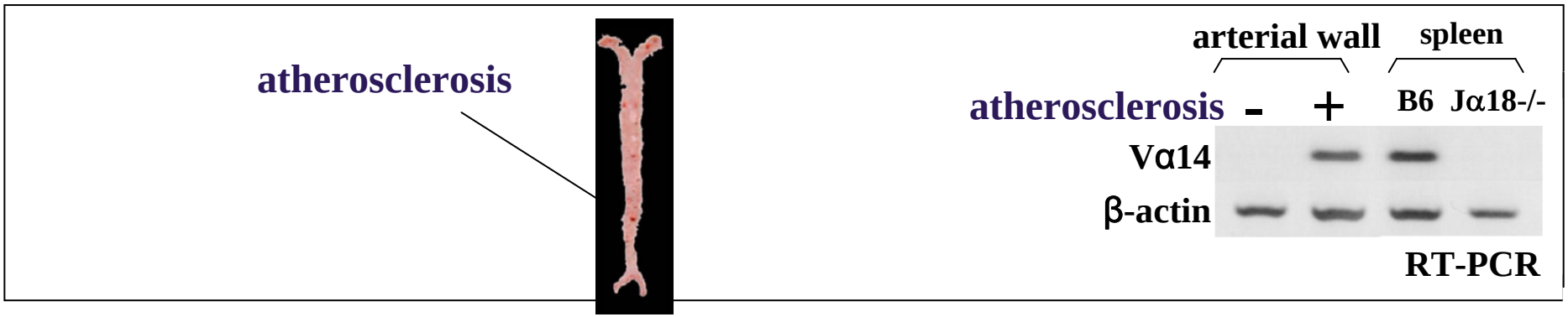


Fig. 9

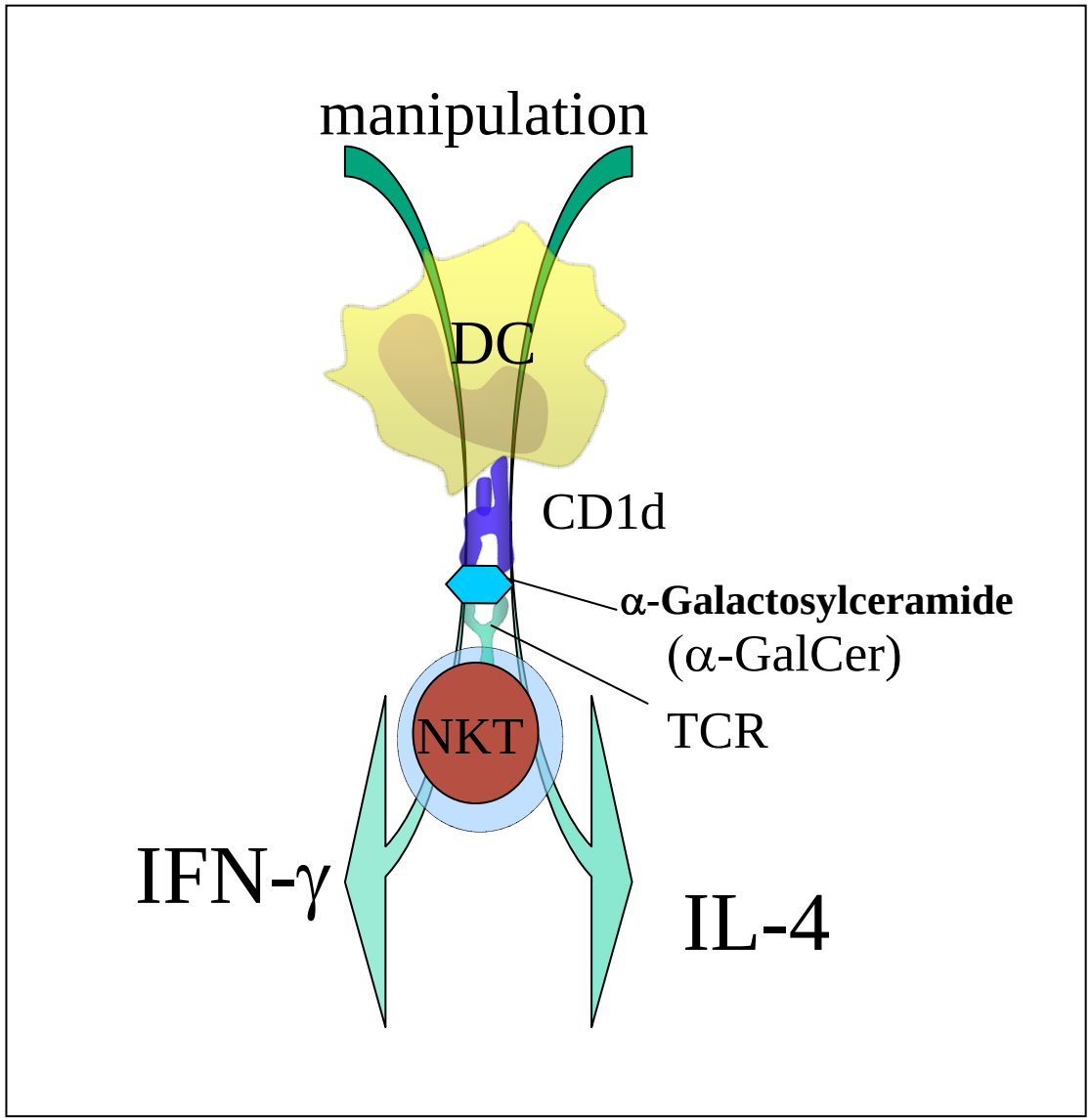

manipulation
DC
CD1d
α-Galactosylceramide
(α-GalCer)
TCR
NKT
IFN-γ
IL-4

Fig. 10

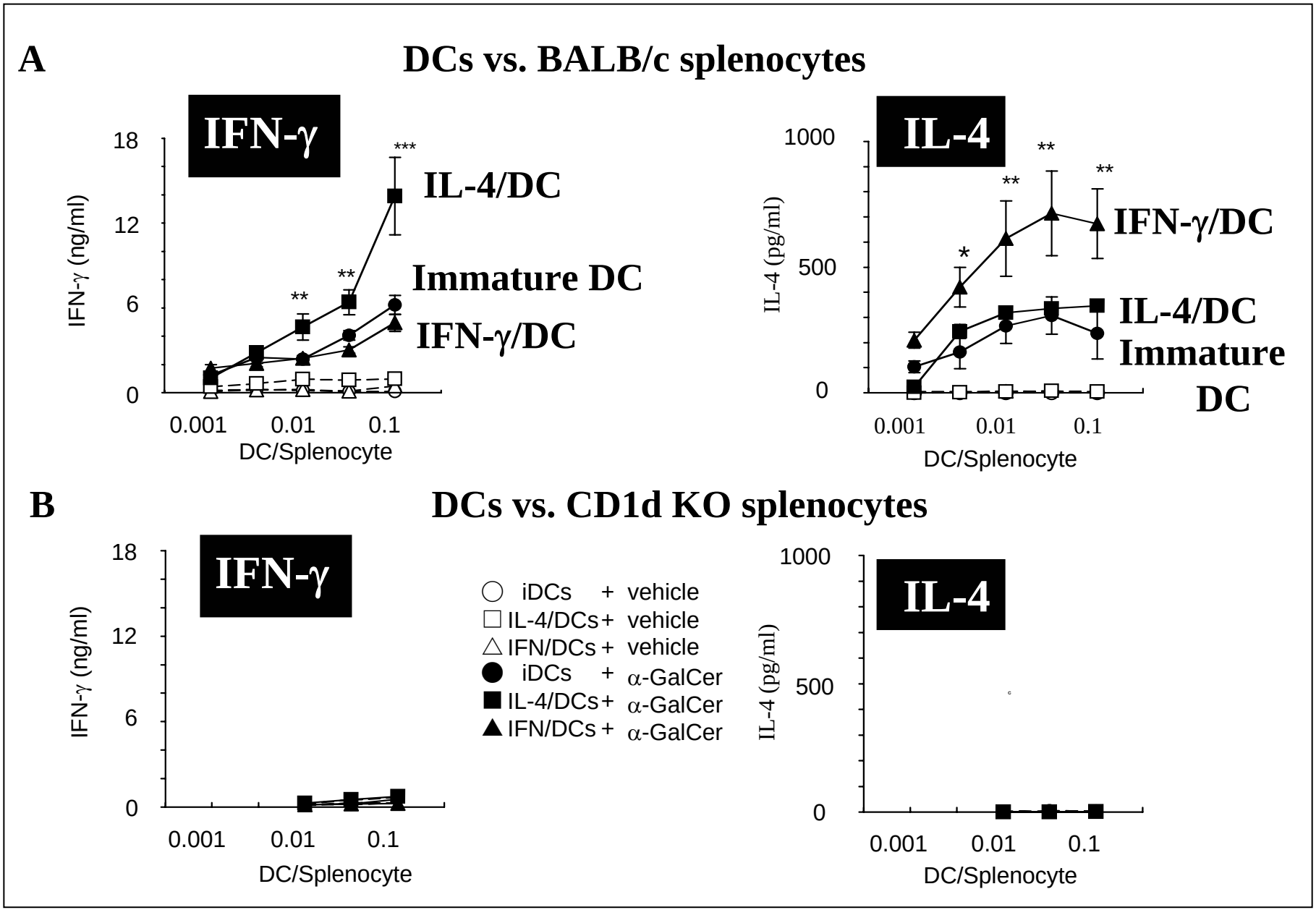

A
DCs vs. BALB/c splenocytes
IFN-γ
IFN-γ (ng/ml)
18
12
6
0
0.001
0.01
0.1
DC/Splenocyte
IL-4/DC
Immature DC
IFN-γ/DC
IL-4
IL-4 (pg/ml)
1000
500
0
IFN-γ/DC
IL-4/DC
Immature DC
B
DCs vs. CD1d KO splenocytes
iDCs + vehicle
IL-4/DCs + vehicle
IFN/DCs + vehicle
iDCs + α-GalCer
IL-4/DCs + α-GalCer
IFN/DCs + α-GalCer

Fig. 11

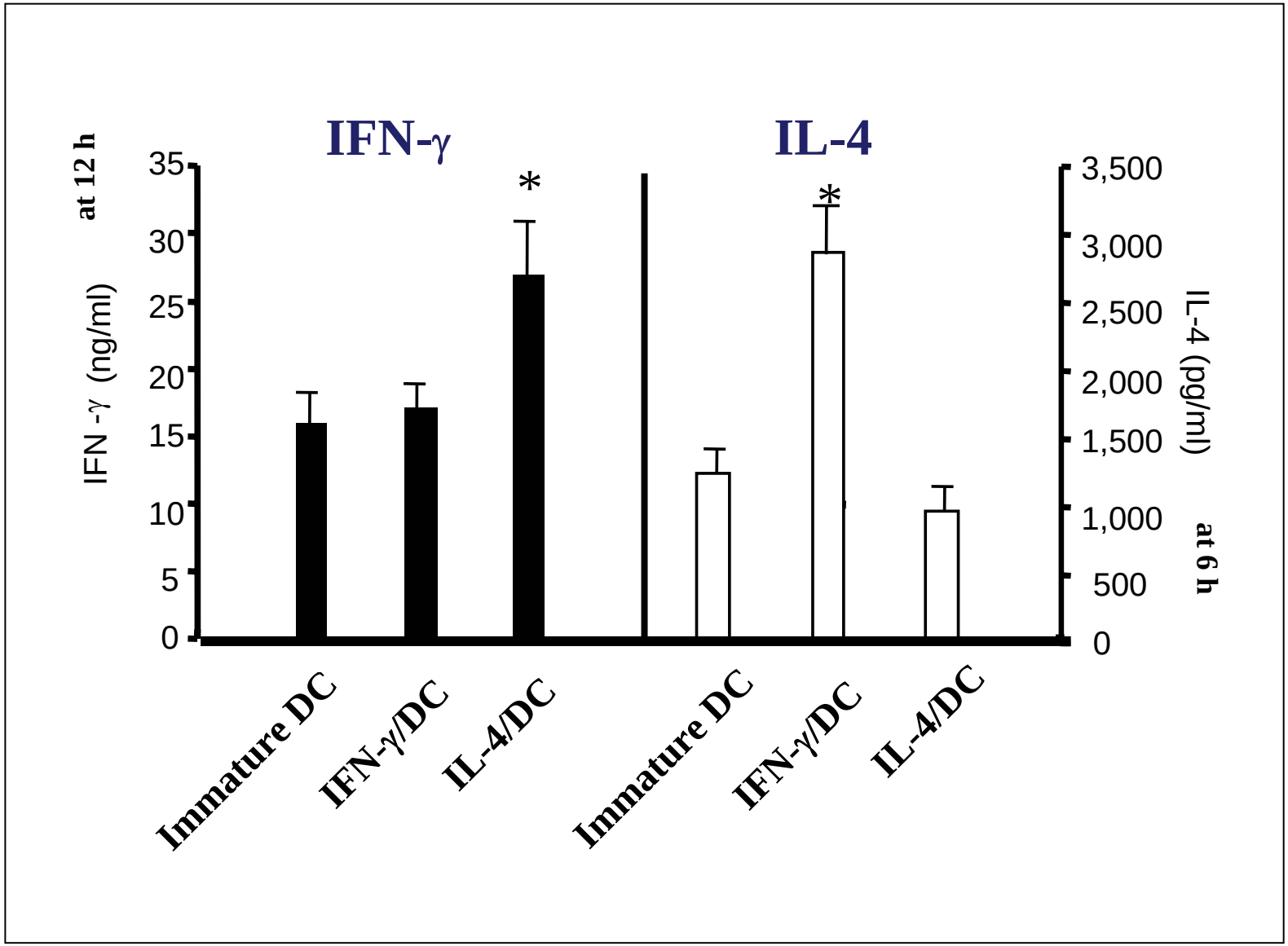

IFN-γ
IL-4
IFN -γ (ng/ml) at 12 h
IL-4 (pg/ml) at 6 h
35
30
25
20
15
10
5
0
3,500
3,000
2,500
2,000
1,500
1,000
500
0
*
*
Immature DC
IFN-γ/DC
IL-4/DC
Immature DC
IFN-γ/DC
IL-4/DC

Fig. 12

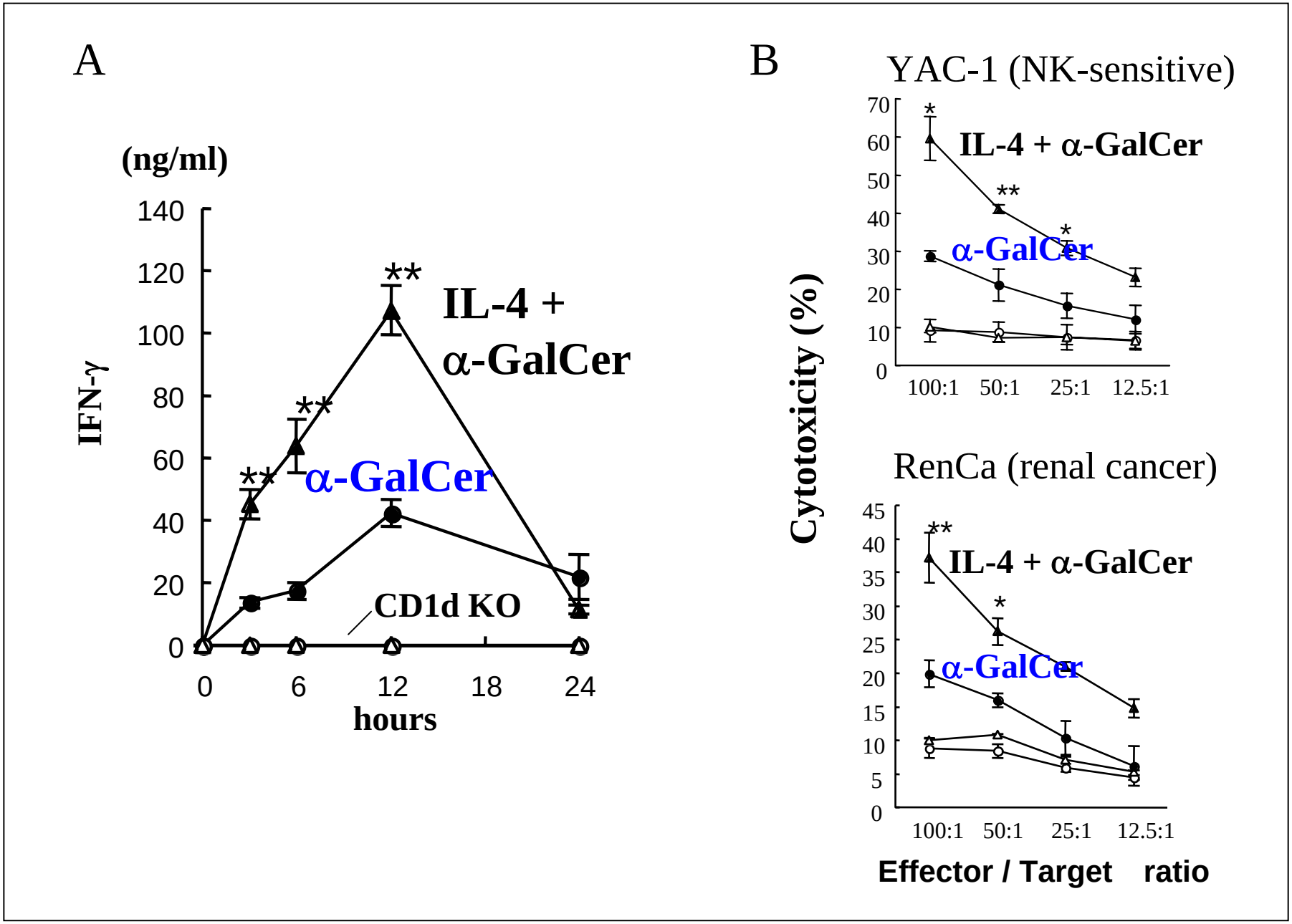

A
(ng/ml)
IFN-γ
140
120
100
80
60
40
20
0
0
6
12
18
24
hours
**
**
**
IL-4 +
α-GalCer
α-GalCer
CD1d KO
B
YAC-1 (NK-sensitive)
Cytotoxicity (%)
70
60
50
40
30
20
10
0
*
**
*
IL-4 + α-GalCer
α-GalCer
100:1
50:1
25:1
12.5:1
RenCa (renal cancer)
45
40
35
30
25
20
15
10
5
0
**
*
IL-4 + α-GalCer
α-GalCer
100:1
50:1
25:1
12.5:1
Effector / Target ratio

Fig. 13

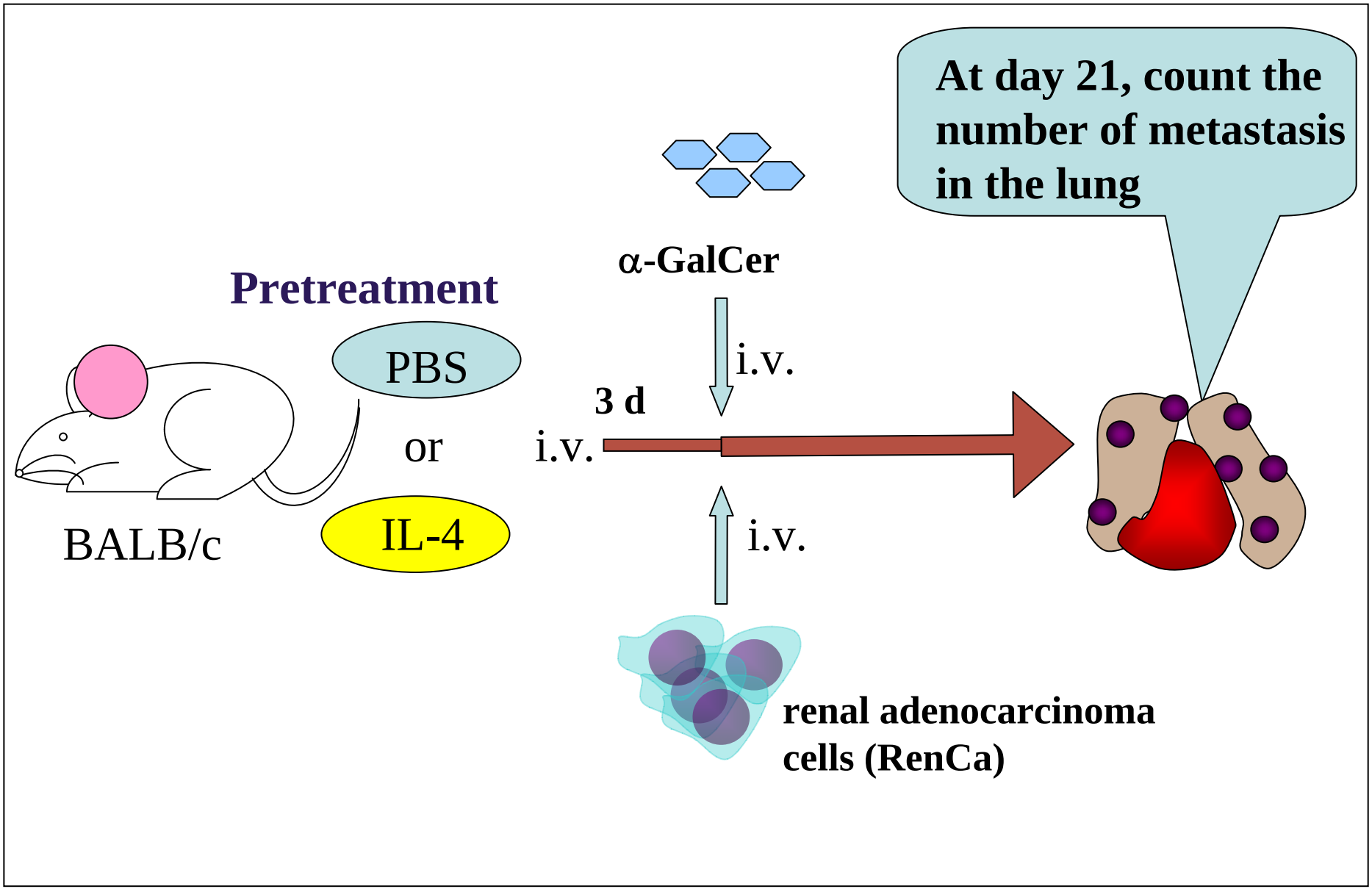
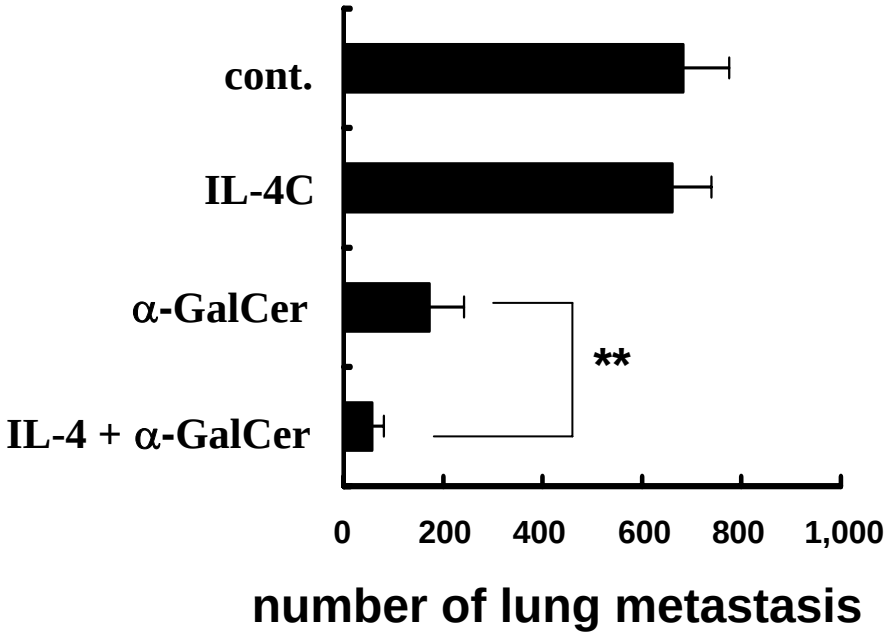

At day 21, count the number of metastasis in the lung
α-GalCer
Pretreatment
PBS
i.v.
3 d
i.v.
or
IL-4
i.v.
BALB/c
renal adenocarcinoma cells (RenCa)

Fig. 14

A


cont.
IL-4C
α-GalCer
IL-4 + α-GalCer
**
0
200
400
600
800
1,000
number of lung metastasis

B

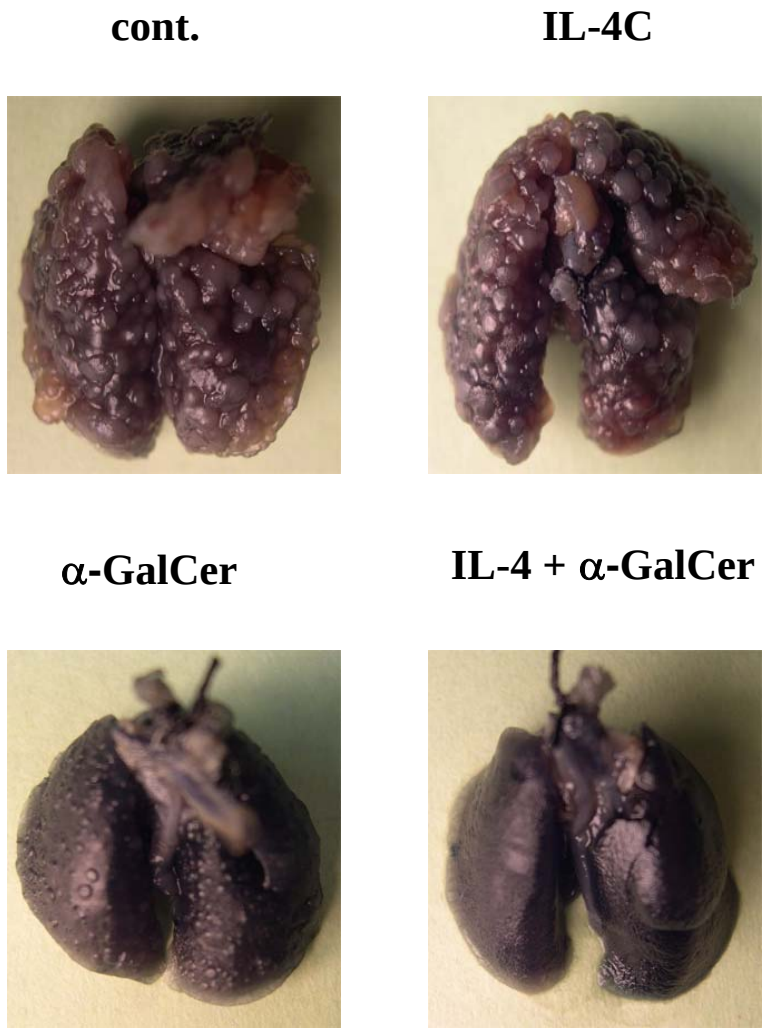

cont.
IL-4C
α-GalCer
IL-4 + α-GalCer

Fig. 15

Negative feedback regulation

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.**

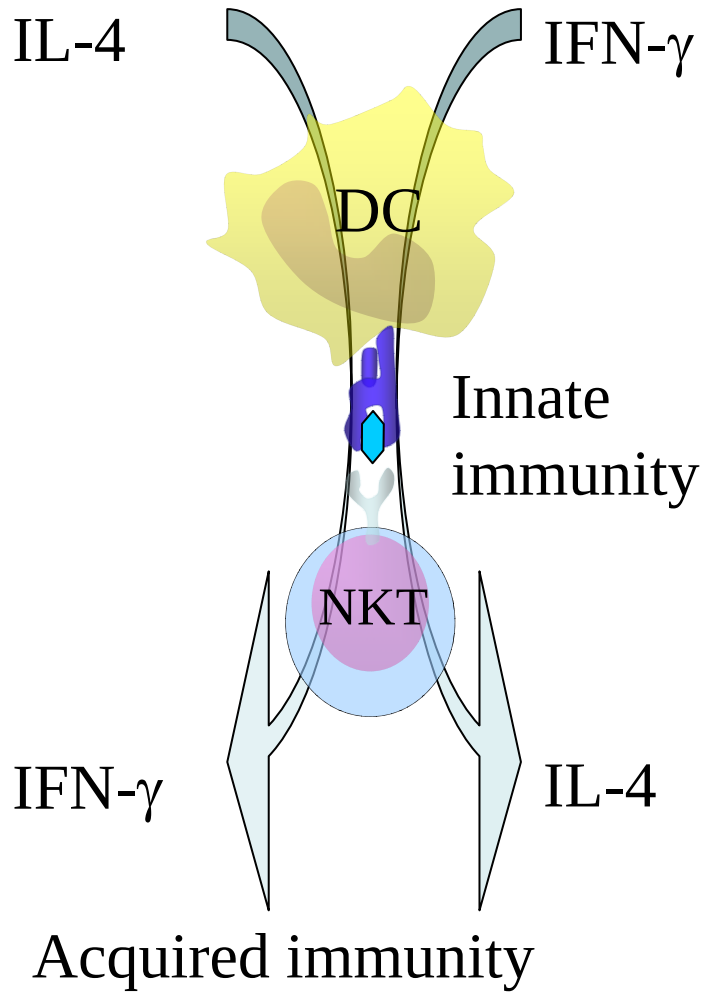


Fig. 16