

Role of α -Galactosylceramide-activated V α 14 Natural Killer T Cells in the Regulation of Allergic Diseases

Chiaki Iwamura¹ and Toshinori Nakayama¹

ABSTRACT

V α 14 natural killer T (NKT) cells produce large amounts of both IL-4 and IFN- γ upon stimulation with a ligand, α -galactosylceramide (α -GalCer), and play a crucial role in various immune responses, including allergic reactions. Interestingly, V α 14 NKT cells are not essential for the induction of specific IgE response but they instead tend to induce suppression of specific IgE upon α -GalCer activation *in vivo*. The suppression in the IgE production is not detected either in V α 14 NKT cell-deficient mice or in IFN- γ -deficient mice. Therefore, activated V α 14 NKT cells are able to exert a potent suppressive activity on Th2 cell differentiation and subsequent IgE production by producing a large amount of IFN- γ . In an OVA-induced asthma model, α -GalCer administration inhibited airway inflammation and airway hyperreactivity by IFN- γ from activated V α 14 NKT cells, thus suggesting the negative regulation of Th2-responses by the activated V α 14 NKT cells.

KEY WORDS

IFN- γ , IgE, Th2 responses, V α 14 NKT cell, α -Galactosylceramide

INTRODUCTION

V α 14 natural killer T (NKT) cells belong to a novel lymphoid lineage distinct from T cells, B cells or NK cells, and they are characterized by the expression of a single invariant antigen receptor encoded by V α 14 and J α 281 segments in association with a highly skewed set of V β s, mainly V β 8.2.¹⁻³ The invariant V α 14/V β 8.2 receptor is not expressed on conventional T cells and its expression is essential for the development of V α 14 NKT cells. In fact, the deletion of the J α 281 gene segment results in the selective loss of NKT cell development (NKT-deficient mice),⁴ while the transgene of the invariant V α 14/V β 8.2 into recombination-activating gene-deficient mice leads to the development of only NKT cells without other lymphoid populations (NKT mice),⁵ thus suggesting the existence of a unique antigen receptor only for NKT cells, but not for conventional T cells. The most potent ligand for the invariant V α 14 NKT antigen receptor is a glycolipid, α -galactosylceramide (α -GalCer), which is exclusively presented by CD1d, a class Ib molecule monomorphic in nature.^{6,7} V α 14 NKT cells are known to play critical roles in infectious diseases⁸

and in the regulation of immune responses, such as in the maintenance of transplantation tolerance, the inhibition of tumor development, and protection against autoimmune disease development.^{1,9} We herein review the role of V α 14 NKT cells in the regulation of Th2 cell differentiation, Th2 responses and the development of allergic asthma.

REGULATION OF TH1 AND TH2 CELL DIFFERENTIATION

Mouse CD4⁺ T cells can be divided into two distinct subpopulations based on their cytokine production pattern, and they are designed as IFN- γ producing Th1, and IL-4 producing Th2 cells.¹⁰ The development of Th1 and Th2 cells is central to the diversity of CD4 T cell-dependent immune responses in infectious, allergic and autoimmune diseases. Th1 cells mediate delayed-type hypersensitivity and organ-specific autoimmune diseases, whereas Th2 cells are involved in the development of allergies and in the defense against extracellular microorganisms.

Th1 and Th2 cells are thought to differentiate from a common precursor and the direction of Th cell differentiation into Th1 and Th2 cells is dependent on

¹Department of Immunology, Graduate School of Medicine, Chiba University, Chiba, Japan.

Correspondence: Dr. Toshinori Nakayama, Department of Immunology (H3), Graduate School of Medicine, Chiba University, 1-8-

1 Inohana, Chuo-ku, Chiba 260-8670, Japan.

Email: tnakayama@faculty.chiba-u.jp

Received 10 October 2006.

©2007 Japanese Society of Allergology

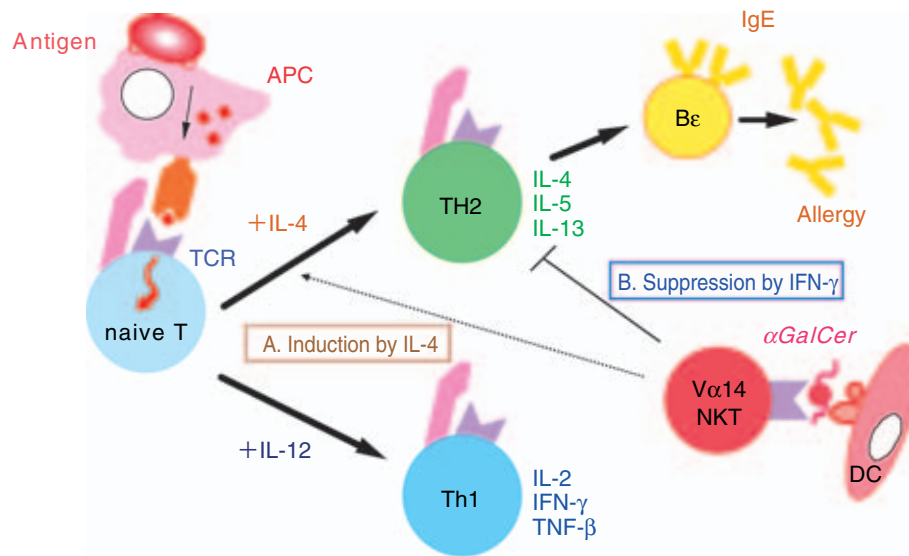


Fig. 1 Two possible roles of $V\alpha 14$ NKT cells in Th1/Th2 cell differentiation. Naïve CD4 T cell differentiate into Th1 or Th2 cells after antigen recognition by TCR in the presence of an appropriate cytokine, such as IL-4 for Th2 cells and IL-12 for Th1 cells. As for the role of activated NKT cells, two possible regulation of the Th1/Th2 cell differentiation can be considered. (A) IL-4 produced by NKT cells induces Th2 differentiation. (B) IFN- γ produced by NKT cells suppresses Th2 differentiation.

the exogenous cytokines. IL-12 is a potent inducer of Th1 cells. $V\alpha 14$ NKT cells are a primary target of IL-12 and they produce only IFN- γ upon IL-12 stimulation. This strongly suggests that $V\alpha 14$ NKT cells function as an inducer of Th1 cells, thus exerting a suppressive activity on the generation of Th2 cells via the production of IFN- γ under certain conditions. On the other hand, IL-4 is required for the differentiation of naïve T cells into Th2 effector cells.¹¹ In contrast to Th1 and Th2 cells with a restricted ability to produce particular cytokines, $V\alpha 14$ NKT cells produce large amounts of both IFN- γ and IL-4 after stimulation with α -GalCer.⁵ As IL-4 and IFN- γ have an opposite effect on Th2 cell differentiation, several mechanisms in the regulation of Th2 cell differentiation by $V\alpha 14$ NKT cells have so far been reported (Fig. 1). Yoshimoto *et al.* have demonstrated that IL-4 produced by NKT cells to induce Th2 cell differentiation (see Fig. 1, A. Induction by IL-4).^{12,13} In contrast, other reports by several investigators have demonstrated that CD1d-deficient or $\beta 2$ -microglobulin-deficient mice, with a small number of NKT cells produce IgE upon immunization with anti-IgD antibody at equivalent levels to that in normal mice.¹⁴⁻¹⁷ A recent analysis suggested that some NK1.1⁺TCR β ⁺ cells in certain lymphoid organs, such as bone marrow or lymph node, were not CD1d-dependent or $V\alpha 14$ J $\alpha 281$ -negative.¹⁸⁻²⁰ Although the NK1.1⁺TCR β ⁺ subpopulation may be a cell source of IL-4, the activation requirements and function of the NK1.1⁺TCR β ⁺ subpopulation have not

been well analyzed. It is therefore important to evaluate the functional role of $V\alpha 14$ NKT cells in the regulation of immune responses, particularly Th2 differentiation and the subsequent IgE antibody responses.

SUPPRESSION OF IgG PRODUCTION BY ACTIVATED $V\alpha 14$ NKT CELLS

To investigate the role of $V\alpha 14$ NKT cells in the regulation of IgE antibody responses, J $\alpha 281$ -deficient ($V\alpha 14$ NKT-deficient) mice were established where the development of $V\alpha 14$ NKT cells was dramatically inhibited.⁴ $V\alpha 14$ NKT-deficient mice were infected with *Nippostrongylus brasiliensis* (Nb), and then were immunized with DNP-conjugated Nb in alum for the induction of DNP-specific IgE production. Equivalent levels of total IgE and DNP-specific IgE, compared to the wild-type mice, were detected in $V\alpha 14$ NKT-deficient mice, where no primary IL-4 was produced. In addition, the DNP-specific IgG1 and IgG2a levels in $V\alpha 14$ NKT-deficient mice were also comparable. These results indicated that $V\alpha 14$ NKT cells were not indispensable for the antigen-specific IgE responses induced by Nb infection immunization.²¹

It has been well documented that the IgE and IgG1 responses are mediated by antigen-specific Th2 cells, and the IgG2a responses depend on Th1 cells. Consequently, we activated $V\alpha 14$ NKT cells *in vivo* with α -GalCer, and the antigen-specific IgE, IgG1 or IgG2a production was thus assessed. The anti-DNP IgE response induced by DNP-OVA immunization dramati-

cally decreased in wild-type mice after α -GalCer injection, whereas no suppression was observed in the NKT-deficient mice. In the anti-DNP-IgG2a responses, however, a significant increase was observed. These results indicated that the stimulation of V α 14 NKT cells with α -GalCer suppressed the antigen-specific Th2 responses, thus resulting in the decreased IgE with either an intact or somewhat enhanced Th1-dependent IgG2a production. In contrast, IL-4 produced by V α 14 NKT cells has little effect on antigen-induced Th2 cell differentiation.²¹

INDUCTION OF TH2 PHENOTYPE BY V α 14 NKT CELL ACTIVATION

Repeated exposure to α -GalCer induced NKT cells to secrete IL-4 but at dramatically reduced levels IFN- γ .^{22,23} Similarly, the immunization of OVA and α -GalCer in complete Freund's adjuvant (CFA) efficiently induced IgE response.²⁴ We injected 2 μ g/mouse α -GalCer after OVA priming in alum 3 times.²¹ This protocol was used because a potent anti-tumor effect by V α 14 NKT cells was observed in an experimental liver metastasis model of B16 melanoma.²⁵ Although the data are not shown, similar production profiles of IFN- γ and IL-4 from V α 14 NKT cells were observed in 2, 4 or 10 μ g of α -GalCer injection (unpublished observation). Burdin *et al.*²² observed the Th2-skewed cytokine profile after the repeated administration of 4–5 μ g of α -GalCer. Singh *et al.*²⁴ used 4 μ g of α -GalCer in CFA, by which α -GalCer may stimulate V α 14 NKT cells repeatedly. It is thus conceivable that the discrepancy between our results and those of the other two groups is due to the differences in the protocol of α -GalCer administration.

ESSENTIAL ROLE OF IFN- γ IN THE REGULATION OF IgE RESPONSES BY THE ACTIVATED V α 14 NKT CELLS

We extended our analysis using IFN- γ -deficient mice and examined whether the effector molecule of the V α 14 NKT cell mediated suppression of IgE response is IFN- γ .²¹ IFN- γ -deficient mice were immunized with OVA in alum after α -GalCer injection, and then the primary IgE and IgG1 responses and secondary IgE response were assessed. As we expected, no suppression in the reduction of IgE was observed in either the primary or secondary response in IFN- γ -deficient mice. In addition, the IgG1 response was not impaired. NKT cells in IFN- γ -deficient mice produced an equivalent level of IL-4 upon stimulation with α -GalCer. Therefore, the suppressive effect on the production of IgE appears to be mediated by IFN- γ produced by V α 14 NKT cells (see Fig. 1, B. Suppression by IFN- γ).

A suppressive effect on IgE production by IFN- γ was reported in several other experimental systems.²⁶⁻³¹ IFN- γ produced by $\gamma\delta$ T cells suppressed the IgE responses in OVA-specific responses³⁰ and

the cutaneous contact sensitivity system.³¹ In addition, since IFN- γ is known to also be produced by CD8 $^+$ $\alpha\beta$ TCR T cells, a possible inhibitory role for these cells in the regulation of IgE responses has also been reported.³²

When V α 14 NKT cells were found to produce IFN- γ after activation with α -GalCer, a unique role of IL-12 was reported.^{33,34} IL-12 is shown to be produced by dendritic cells only when they interacted with α -GalCer-activated V α 14 NKT cells. The IL-12 in turn enhanced the IFN- γ production of the activated V α 14 NKT cells. As a result, IL-12 may play a significant role in the IFN- γ -mediated suppressive effect on the IgE responses.

INHIBITION OF TH2 CELL DIFFERENTIATION BY ACTIVATED V α 14 NKT CELLS IN VITRO

The role of ligand-activated V α 14 NKT cells on Th2 cell differentiation was examined more precisely through the use of an *in vitro* induction culture system.³⁵ Naïve CD4 T cell obtained from (B6 \times BALB/c) F1 mice were stimulated with immobilized anti-TCR mAb in the presence of IL-4 to allow Th2 cell differentiation *in vitro*. V α 14 NKT cells from α -GalCer-treated V α 14 NKT mice with B6 background were added to the induction culture, and the intracellular production of IFN- γ and IL-4 in K d -positive T cells was assessed (Fig. 2). In this culture system, an IL-4 dose-dependent increase in the generation of Th2 cells was observed. However, the addition of activated V α 14 NKT cells in the induction culture inhibited IL-4-producing Th2 cell differentiation. In addition, the number of IFN- γ -producing Th1 cell differentiation was significantly enhanced. These results clearly indicated that Th2 cell differentiation was inhibited by the addition of activated V α 14 NKT cells. Moreover, the V α 14 NKT cell-mediated inhibition of Th2 cell differentiation was blocked by the addition of anti-IFN- γ mAb. Therefore, similar to the mechanisms governing IgE suppression in an *in vivo* experimental system, IFN- γ thus appear to be an effector molecule for the inhibition of Th2 cell differentiation induced by activated V α 14 NKT cells *in vitro* (see Fig. 1, B. Suppression by IFN- γ).

REGULATION OF AIRWAY INFLAMMATION BY ACTIVATED V α 14 NKT CELLS

A suppressive effect of INF- γ from activated V α 14 NKT cells has been reported in several experimental murine asthma model.³⁶⁻³⁸ Matsuda *et al.* showed that a single administration of α -GalCer almost completely abrogated the infiltration of eosinophils in the lung and reduced airway hyperreactivity (AHR), together with the decreased Th2 cytokine expression in BALF and decreased goblet cell hyperplasia.³⁶ This protection was accompanied by a significant increase in the serum levels of antigen-specific IgG2a and a decrease

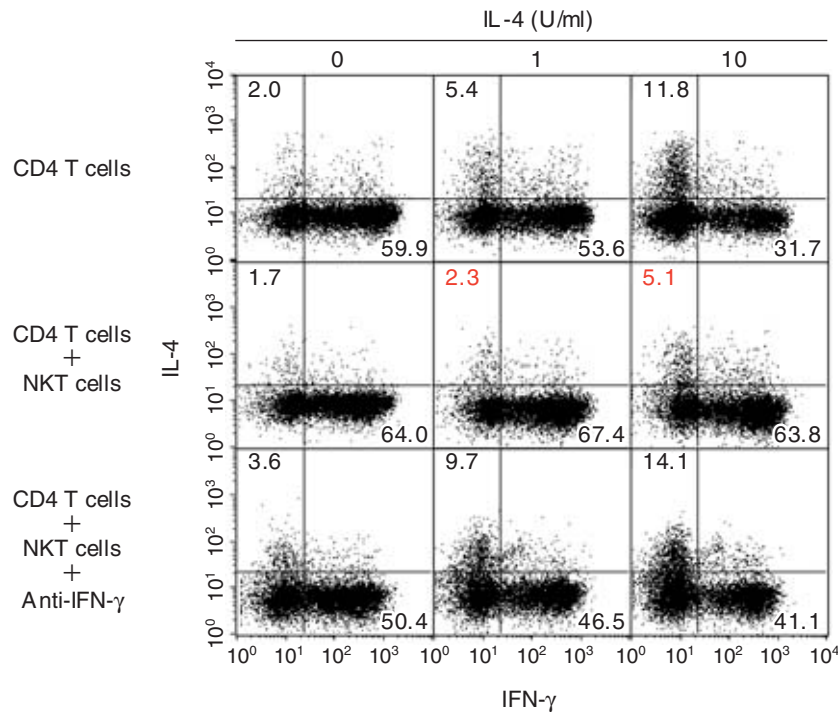


Fig. 2 Inhibition of Th2 cell differentiation by α -GalCer-activated V α 14 NKT cells. Naïve CD4 T cells (1.5×10^6) from (B6 \times BALB/c) F1 mice were stimulated with immobilized anti-TCR mAb (H57-597; 30 mg/ml) in the presence of 1 or 10 U/ml of recombinant IL-4. Activated V α 14 NKT cells (0.75×10^6) from NKT mice with α -GalCer treatment were added at the beginning of the induction culture. Where indicated, anti-IFN- γ mAb (5 μ g/ml) was added to the induction culture. Intracellular staining profiles of IL-4 and IFN- γ of electronically gated K d -bearing T cells are shown. The percentages of cells present in each area indicated.

in those of antigen-specific IgE. This inhibitory effect by α -GalCer administration was not observed in IFN- γ KO mice. Furthermore, Hachem *et al.* demonstrated the role of IFN- γ from NKT cells in the protection of allergic asthma was shown by means of an adoptive transfer system.³⁷ The adoptive transfer of NKT cells from OVA-sensitized and α -GalCer-treated mice suppressed the OVA-induced AHR and airway inflammation in recipient mice. This protective effect was abolished by the transfer of NKT cells from IFN- γ KO mice, thus indicating that IFN- γ produced by NKT cells is required for the transfer of the inhibiting effects of eosinophilia and AHR. These data suggest that the specific activation of NKT cells by α -GalCer inhibits the antigen-specific Th2 responses in the lung and AHR, possibly by IFN- γ production.

In contrast, the requirement for NKT cells in the development of the characteristic features of asthma has been reported.^{39,40} Akbari *et al.* demonstrated that V α 14 NKT cell-deficient mice were shown to develop decreased AHR and OVA induced-airway inflammation, and the adoptive transfer of V α 14 NKT cells producing IL-4 and IL-13 then completely re-

stored them.³⁹ They concluded that V α 14 NKT cells in the lung play a critical role in the development of asthma, thus suggesting that the suppression of V α 14 NKT cell function might be a therapeutic strategy for the treatment of asthma. They also proposed the involvement of unknown self-antigens, which are exposed during antigen challenge into the lung and bind to CD1d, because OVA itself is unable to activate V α 14 NKT cells.

CONCLUSIONS

Since the number of V α 14 NKT cells is dramatically reduced in the thymus and periphery in the V α 14 NKT-deficient mice, we conclude that V α 14 NKT cells and their IL-4 production are not essential for antigen-specific Th2 cell differentiation and the subsequent IgE response induced by Nb infection and OVA immunization. More interestingly, a unique regulatory role of V α 14 NKT cells on Th2 cell differentiation and a selective *in vivo* suppression of IgE production in mice treated with α -GalCer during OVA priming or Nb infection have been reported. OVA-induced airway inflammation and AHR were sup-

pressed by the activated V α 14 NKT cells,³⁶⁻³⁸ whereas the development of airway inflammation was dependent on the presence of V α 14 NKT cells.³⁹ These reports appear to be contradictory, and thus, a more comprehensive analysis is required to establish an optimal therapeutic strategy for allergic asthma using V α 14 NKT cells as a target. Recently, new endogenous and exogenous ligands that stimulate V α 14 NKT cells through distinct mechanisms independent of α -GalCer have been reported.^{41,42} We need to await the investigation on the involvement of these new ligands in the Th2 immune responses and allergic diseases. In any event, V α 14 NKT cells (V α 24 NKT cells in human) may be an intriguing target for establishing a new strategy for the treatment of allergic diseases.

ACKNOWLEDGEMENTS

This work was supported by grants from the Ministry of Education, Culture, Sports, Science and Technology (Japan) (Grants-in-Aid for: Scientific Research in Priority Areas #17016010 and #17047007; Scientific Research B #17390139, Scientific Research C #18590466; Grant-in-Aid for Young Scientists #17790318 and: Special Coordination Funds for Promoting Science and technology), the Ministry of Health, Labor and Welfare (Japan), The Japan Health Science Foundation, Kanae Foundation and Uehara Memorial Foundation and Mochida Foundation.

REFERENCES

1. Taniguchi M, Harada M, Kojo S *et al.* The regulatory role of Valpha14 NKT cells in innate and acquired immune response. *Annu. Rev. Immunol.* 2003;**21**:483-513.
2. Godfrey DI, MacDonald HR, Kronenberg M *et al.* NKT cells: what's in a name? *Nat. Rev. Immunol.* 2004;**4**:231-237.
3. Kronenberg M. Toward an understanding of NKT cell biology: progress and paradoxes. *Annu. Rev. Immunol.* 2005;**23**:877-900.
4. Cui J, Shin T, Kawano T *et al.* Requirement for Valpha14 NKT cells in IL-12-mediated rejection of tumors. *Science* 1997;**278**:1623-1626.
5. Kawano T, Cui J, Koezuka Y *et al.* CD1d-restricted and TCR-mediated activation of valpha14 NKT cells by glycosylceramides. *Science* 1997;**278**:1626-1629.
6. Godfrey DI, Kronenberg M. Going both ways: immune regulation via CD1d-dependent NKT cells. *J. Clin. Invest.* 2004;**114**:1379-1388.
7. Brigl M, Brenner MB. CD1: antigen presentation and T cell function. *Annu. Rev. Immunol.* 2004;**22**:817-890.
8. Taniguchi M, Seino K, Nakayama T. The NKT cell system: bridging innate and acquired immunity. *Nat. Immunol.* 2003;**4**:1164-1165.
9. Smyth MJ, Godfrey DI, Trapani JA. A fresh look at tumor immunosurveillance and immunotherapy. *Nat. Immunol.* 2001;**2**:293-299.
10. Mosmann TR, Cherwinski H, Bond MW *et al.* Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J. Immunol.* 1986;**136**:2348-2357.
11. Paul WE. Interleukin-4: a prototypic immunoregulatory lymphokine. *Blood* 1991;**77**:1859-1870.
12. Yoshimoto T, Bendelac A, Hu-Li J *et al.* 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. U. S. A.* 1995;**92**:11931-11934.
13. Yoshimoto T, Bendelac A, Watson C *et al.* Role of NK1.1+ T cells in a TH2 response and in immunoglobulin E production. *Science* 1995;**270**:1845-1847.
14. Smiley ST, Kaplan MH, Grusby MJ. Immunoglobulin E production in the absence of interleukin-4-secreting CD1-dependent cells. *Science* 1997;**275**:977-979.
15. Brown DR, Fowell DJ, Corry DB *et al.* Beta 2-microglobulin-dependent NK1.1+ T cells are not essential for T helper cell 2 immune responses. *J. Exp. Med.* 1996;**184**:1295-1304.
16. Zhang Y, Rogers KH, Lewis DB. Beta 2-microglobulin-dependent T cells are dispensable for allergen-induced T helper 2 responses. *J. Exp. Med.* 1996;**184**:1507-1512.
17. Guery JC, Galbiati F, Smiroldo S *et al.* Selective development of T helper (Th) 2 cells induced by continuous administration of low dose soluble proteins to normal and beta (2)-microglobulin-deficient BALB/c mice. *J. Exp. Med.* 1996;**183**:485-497.
18. Eberl G, Lees R, Smiley ST *et al.* Tissue-specific segregation of CD1d-dependent and CD1d-independent NK T cells. *J. Immunol.* 1999;**162**:6410-6419.
19. Zeng D, Gazit G, Dejbakhsh-Jones S *et al.* Heterogeneity of NK1.1+ T cells in the bone marrow: divergence from the thymus. *J. Immunol.* 1999;**163**:5338-5345.
20. Hammond KJ, Pelikan SB, Crowe NY *et al.* NKT cells are phenotypically and functionally diverse. *Eur. J. Immunol.* 1999;**29**:3768-3781.
21. Cui J, Watanabe N, Kawano T *et al.* Inhibition of T helper cell type 2 cell differentiation and immunoglobulin E response by ligand-activated Valpha14 natural killer T cells. *J. Exp. Med.* 1999;**190**:783-792.
22. Burdin N, Brossay L, Kronenberg M. Immunization with alpha-galactosylceramide polarizes CD1-reactive NK T cells towards Th2 cytokine synthesis. *Eur. J. Immunol.* 1999;**29**:2014-2025.
23. Harada M, Seino K, Wakao H *et al.* Down-regulation of the invariant Valpha14 antigen receptor in NKT cells upon activation. *Int. Immunol.* 2004;**16**:241-247.
24. Singh N, Hong S, Scherer DC *et al.* Cutting edge: activation of NK T cells by CD1d and alpha-galactosylceramide directs conventional T cells to the acquisition of a Th2 phenotype. *J. Immunol.* 1999;**163**:2373-2377.
25. Kawano T, Cui J, Koezuka Y *et al.* Natural killer-like non-specific tumor cell lysis mediated by specific ligand-activated Valpha14 NKT cells. *Proc. Natl. Acad. Sci. U. S. A.* 1998;**95**:5690-5693.
26. Ferrick DA, Schrenzel MD, Mulvania T *et al.* Differential production of interferon-gamma and interleukin-4 in response to Th1- and Th2-stimulating pathogens by gamma delta T cells *in vivo*. *Nature* 1995;**373**:255-257.
27. Zuany-Amorim C, Ruffie C, Haile S *et al.* Requirement for gammadelta T cells in allergic airway inflammation. *Science* 1998;**280**:1265-1267.
28. Coffman RL, Carty J. A T cell activity that enhances polyclonal IgE production and its inhibition by interferon-gamma. *J. Immunol.* 1986;**136**:949-954.
29. Gajewski TF, Joyce J, Fitch FW. Antiproliferative effect of IFN-gamma in immune regulation. III. Differential selection of TH1 and TH2 murine helper T lymphocyte clones using recombinant IL-2 and recombinant IFN-gamma. *J.*

- Immunol.* 1989;**143**:15-22.
30. McMenamin C, Pimm C, McKersey M *et al.* Regulation of IgE responses to inhaled antigen in mice by antigen-specific gamma delta T cells. *Science* 1994;**265**:1869-1871.
 31. Szczepanik M, Anderson LR, Ushio H *et al.* Gamma delta T cells from tolerized alpha beta T cell receptor (TCR)-deficient mice inhibit contact sensitivity-effector T cells *in vivo*, and their interferon-gamma production *in vitro*. *J. Exp. Med.* 1996;**184**:2129-2139.
 32. Romagnani S. Induction of TH1 and TH2 responses: a key role for the 'natural' immune response? *Immunol. Today* 1992;**13**:379-381.
 33. Kitamura H, Iwakabe K, Yahata T *et al.* The natural killer T (NKT) cell ligand alpha-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.* 1999;**189**:1121-1128.
 34. Tomura M, Yu WG, Ahn HJ *et al.* A novel function of Valpha14⁺CD4⁺NKT cells: stimulation of IL-12 production by antigen-presenting cells in the innate immune system. *J. Immunol.* 1999;**163**:93-101.
 35. Yamashita M, Kimura M, Kubo M *et al.* T cell antigen receptor-mediated activation of the Ras/mitogen-activated protein kinase pathway controls interleukin 4 receptor function and type-2 helper T cell differentiation. *Proc. Natl. Acad. Sci. U. S. A.* 1999;**96**:1024-1029.
 36. Matsuda H, Suda T, Sato J *et al.* alpha-Galactosylceramide, a ligand of natural killer T cells, inhibits allergic airway inflammation. *Am. J. Respir. Cell Mol. Biol.* 2005;**33**:22-31.
 37. Hachem P, Lisbonne M, Michel ML *et al.* Alpha-galactosylceramide-induced iNKT cells suppress experimental allergic asthma in sensitized mice: role of IFN-gamma. *Eur. J. Immunol.* 2005;**35**:2793-2802.
 38. Morishima Y, Ishii Y, Kimura T *et al.* Suppression of eosinophilic airway inflammation by treatment with alpha-galactosylceramide. *Eur. J. Immunol.* 2005;**35**:2803-2814.
 39. Akbari O, Stock P, Meyer E *et al.* Essential role of NKT cells producing IL-4 and IL-13 in the development of allergen-induced airway hyperreactivity. *Nat. Med.* 2003;**9**:582-588.
 40. Meyer EH, Goya S, Akbari O *et al.* Glycolipid activation of invariant T cell receptor⁺ NK T cells is sufficient to induce airway hyperreactivity independent of conventional CD4⁺ T cells. *Proc. Natl. Acad. Sci. U. S. A.* 2006;**103**:2782-2787.
 41. Kinjo Y, Wu D, Kim G *et al.* Recognition of bacterial glycosphingolipids by natural killer T cells. *Nature* 2005;**434**:520-525.
 42. Mattner J, Debord KL, Ismail N *et al.* Exogenous and endogenous glycolipid antigens activate NKT cells during microbial infections. *Nature* 2005;**434**:525-529.