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REVIEW ARTICLE

Role of α-Galactosylceramide-activated Vα14 Natural Killer T Cells in the Regulation of Allergic Diseases

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ABSTRACT

Vα14 natural killer T (NKT) cells produce large amounts of both IL-4 and IFN-γ upon stimulation with a ligend, α-galactosylceramide (α-GalCer), and play a crucial role in various immune responses, including allergic reactions. Interestingly, Vα14 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 Vα14 NKT cell-deficient mice or in IFN-γ-deficient mice. Therefore, activated Vα14 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 Vα14 NKT cells, thus suggesting the negative regulation of Th2-responses by the activated Vα14 NKT cells.

KEY WORDS

IFN-γ, IgE, Th2 responses, Vα14 NKT cell, α-Galactosylceramide

INTRODUCTION

Vα14 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 Vα14 and Jα281 segments in association with a highly skewed set of Vβs, mainly Vβ8.2.[1-3] The invariant Vα14/Vβ8.2 receptor is not expressed on conventional T cells and its expression is essential for the development of Vα14 NKT cells. In fact, the deletion of the Jα281 gene segment results in the selective loss of NKT cell development (NKT-deficient mice),[4] while the transgene of the invariant Vα14/Vβ8.2 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 Vα14 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] Vα14 NKT cells are known to play critical roles in infectious diseases[8] 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 Vα14 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

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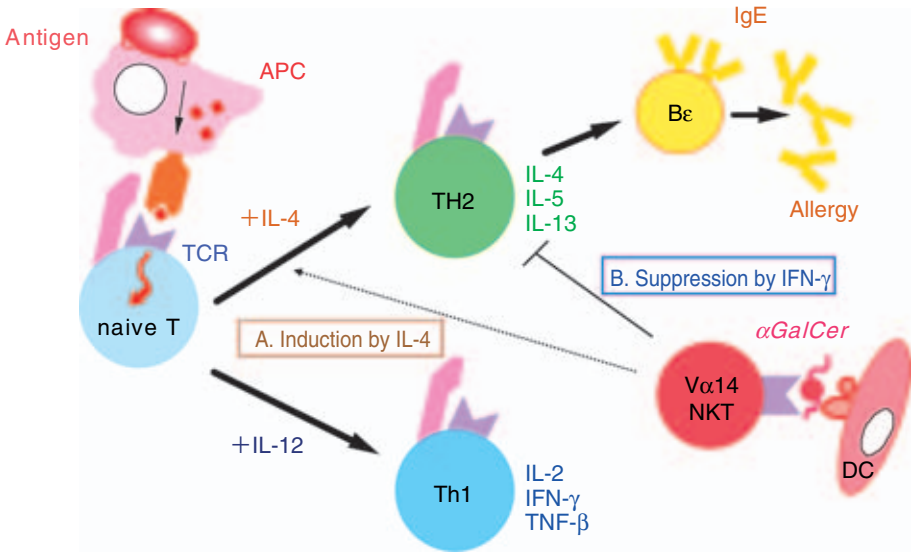

Fig. 1 Two possible roles of Vα14 NKT cells in Th1/Th2 cell differentiation. Naïve CD4 T cell differentiate into Th1 or Th2 cells after antigen recognition by TCR in the presence of an appropriate cytokine, such as IL-4 for Th2 cells and IL-12 for Th1 cells. As for the role of activated NKT cells, two 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. Vα14 NKT cells are a primary target of IL-12 and they produce only IFN-γ upon IL-12 stimulation. This strongly suggests that Vα14 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 effecter cells.[11] In contrast to Th1 and Th2 cells with a restricted ability to produce particular cytokines, Vα14 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 Vα14 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 β2-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$^+$TCRβ$^+$ cells in certain lymphoid organs, such as bone marrow or lymph node, were not CD1d-dependent or Vα14Jα281-negative.[18-20] Although the NK1.1$^+$TCRβ$^+$ subpopulation may be a cell source of IL-4, the activation requirements and function of the NK1.1$^+$TCRβ$^+$ subpopulation have not been well analyzed. It is therefore important to evaluate the functional role of Vα14 NKT cells in the regulation of immune responses, particularly Th2 differentiation and the subsequent IgE antibody responses.

SUPPRESSION OF IgG PRODUCTION BY ACTIVATED Vα14 NKT CELLS

To investigate the role of Vα14 NKT cells in the regulation of IgE antibody responses, Jα281-deficient (Vα14NKT-deficient) mice were established where the development of Vα14 NKT cells was dramatically inhibited.[4] Vα14 NKT-deficient mice were infected with *Nippstrongylus barasilensis* (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 Vα14 NKT-deficient mice, where no primary IL-4 was produced. In addition, the DNP-specific IgG1 and IgG2a levels in Vα14 NKT-deficient mice were also comparable. These results indicated that Vα14 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 Vα14 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 Vα14 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 Vα14 NKT cells has little effect on antigen-induced Th2 cell differentiation.[21]

INDUCTION OF TH2 PHENOTYPE BY Vα14 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 μg/mouse α-GalCer after OVA priming in alum 3 times.[21] This protocol was used because a potent anti-tumor effect by Vα14 NKT cells was observed in an experimental liver matastasis model of B16 melanoma.[25] Although the data are not shown, similar production profiles of IFN-γ and IL-4 from Vα14 NKT cells were observed in 2, 4 or 10 μg of α-GalCer injection (unpublished observation). Burdin *et al.*[22] observed the Th2-skewed cytokine profile after the repeated administration of 4–5 μg of α-GalCer. Singh *et al.*[24] used 4 μg of α-GalCer in CFA, by which α-GalCer may stimulate Vα14 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 Vα14 NKT CELLS

We extended our analysis using IFN-γ-deficient mice and examined whether the effector molecule of the Vα14 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 Vα14 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 γδ T cells suppressed the IgE responses in OVA-specific responses[30] and the cutaneous contact sensitivity system.[31] In addition, since IFN-γ is known to also be produced by $CD8^+$ αβ TCR T cells, a possible inhibitory role for these cells in the regulation of IgE responses has also been reported.[32]

When Vα14 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 Vα14 NKT cells. The IL-12 in turn enhanced the IFN-γ production of the activated Vα14 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 Vα14 NKT CELLS IN VITRO

The role of ligand-activated Vα14 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 × BALB/c) F1 mice were stimulated with immobilized anti-TCR mAb in the presence of IL-4 to allow Th2 cell differentiation *in vitro*. Vα14 NKT cells from α-GalCer-treated Vα14 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 Vα14 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 Vα14 NKT cells. Moreover, the Vα14 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 Vα14 NKT cells *in vitro* (see Fig. 1, B. Suppression by IFN-γ).

REGULATION OF AIRWAY INFLAMMATION BY ACTIVATED Vα14 NKT CELLS

A suppressive effect of INF-γ from activated Vα14 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

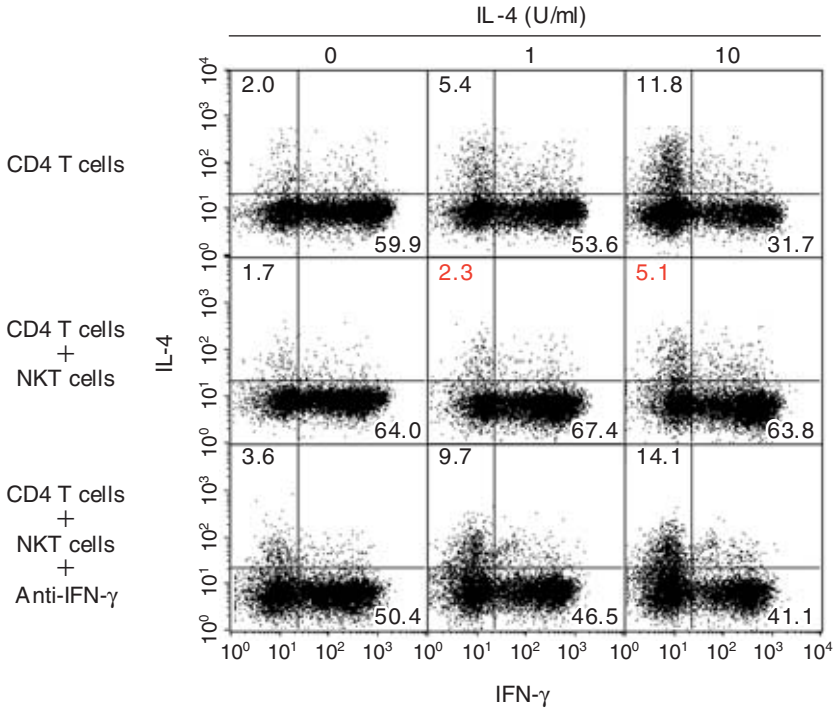

Fig. 2 Inhibition of Th2 cell differentiation by α-GalCer-activated Vα14 NKT cells. Naïve CD4 T cells (1.5×10^6) from (B6×BALB/c) F1 mice were stimulated with immobilized anti-TCR mAb (H57-597; 30 mg/ml) in the presence of 1 or 10 U/ml of recombinant IL-4. Activated Vα14 NKT cells (0.75×10^6) from NKT mice with α-GalCer treatment were added at the beginning of the induction culture.Where indicated, anti-IFN-γ mAb (5 μg/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 Vα14 NKT cell-deficient mice were shown to develop decreased AHR and OVA induced-airway inflammation, and the adoptive transfer of Vα14 NKT cells producing IL-4 and IL-13 then completely restored them.[39] They concluded that Vα14 NKT cells in the lung play a critical role in the development of asthma, thus suggesting that the suppression of Vα14 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 Vα14 NKT cells.

CONCLUSIONS

Since the number of Vα14 NKT cells is dramatically reduced in the thymus and periphery in the Vα14 NKT-deficient mice, we conclude that Vα14 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 Vα14 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 Vα14 NKT cells,[36-38] whereas the development of airway inflammation was dependent on the presence of Vα14 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 Vα14 NKT cells as a target. Recently, new endogenous and exgenous ligands that stimulate Vα14 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, Vα14 NKT cells (Vα24 NKT cells in human) may be an intriguing target for establishing a new strategy for the treatment of allergic diseases.

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