Nonredundant Roles for CD1d-restricted Natural Killer T Cells and Conventional CD4+ T Cells in the Induction of Immunoglobulin E Antibodies in Response to Interleukin 18 Treatment of Mice

Tomohiro Yoshimoto,1,2 Booki Min,3 Takaaki Sugimoto,1,2 Nobuki Hayashi,1,2 Yuriko Ishikawa,1,2 Yuki Sasaki,1,2 Hitomi Hata,1,2 Kazuyoshi Takeda,4 Ko Okumura,4 Luc Van Kaer,5 William E. Paul,3 and Kenji Nakanishi1,2

1Department of Immunology and Medical Zoology, Hyogo College of Medicine, Nishinomiya, Hyogo 663-8501, Japan
2Core Research for Evolutional Science and Technology, Japan Science and Technology Corporation, Saitama 332-0012, Japan
3Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892
4Department of Immunology, Juntendo University School of Medicine, Tokyo 113-8421, Japan
5Department of Microbiology and Immunology, Vanderbilt University School of Medicine, Nashville, TN 37232

Abstract

Interleukin (IL)-18 synergizes with IL-12 to promote T helper cell (Th)1 responses. Somewhat paradoxically, IL-18 administration alone strongly induces immunoglobulin (Ig)E production and allergic inflammation, indicating a role for IL-18 in the generation of Th2 responses. The ability of IL-18 to induce IgE is dependent on CD4+ T cells, IL-4, and signal transducer and activator of transcription (stat)6. Here, we show that IL-18 fails to induce IgE both in CD1d−/− mice that lack natural killer T (NKT) cells and in class II−/− mice that lack conventional CD4+ T cells. However, class II−/− mice reconstituted with conventional CD4+ T cells show the capacity to produce IgE in response to IL-18. NKT cells express high levels of IL-18 receptor (R) chain and produce significant amounts of IL-4, IL-9, and IL-13, and induce CD40 ligand expression in response to IL-2 and IL-18 stimulation in vitro. In contrast, conventional CD4+ T cells express low levels of IL-18Rα and poorly respond to IL-2 and IL-18. Nevertheless, conventional CD4+ T cells are essential for B cell IgE responses after the administration of IL-18. These findings indicate that NKT cells might be the major source of IL-4 in response to IL-18 administration and that conventional CD4+ T cells demonstrate their helper function in the presence of NKT cells.

Key words: IL-18R • CD4+ NK1.1+ T cells • Th2 cytokines • CD40 ligand • allergy

Introduction

IL-18, an IL-1–like cytokine that requires cleavage by caspase-1 to become active, was originally identified as a factor that enhances IFN-γ production by Th1 cells in the presence of anti-CD3 Ab plus IL-12 (1, 2). Later studies have revealed that IL-18 and IL-12 directly and synergistically induce IFN-γ production by Th1 cells, nonpolarized T cells, B cells, NK cells, macrophages, and dendritic cells (3–8). However, our recent studies and those of others have demonstrated that in the absence of IL-12, IL-18 promotes Th2 cytokine production by T cells, basophils, and mast cells (9–13). In the presence of IL-3, IL-18 stimulates basophils and mast cells to produce IL-4 and IL-13 even without FceR cross-linkage (9). CD4+ T cells cultured with IL-2 and IL-18, without TCR engagement, express CD40 ligand (L),* produce IL-4 and IL-13, and induce B cells to secrete IgE in vitro (10). Consistent with these findings, the administration of relatively high doses of IL-18, have demonstrated that in the absence of IL-12, IL-18 promotes Th2 cytokine production by T cells, basophils, and mast cells (9–13).

*Abbreviations used in this paper: α-GalCer, α-galactosylceramide; CFSE, carboxyfluorescein diacetate succinimidyl ester; L, ligand; MFI, mean fluorescence intensity; Tg, transgenic.
which have the ability to induce Th1 diseases when administrated with IL-12 to WT mice (14), results in striking increases in serum IgE that are dependent on CD4 + T cells, IL-4, and signal transducer and activator of transcription 6 but are independent of TCR engagement (10). Transgenic (Tg) mice that overexpress IL-18 or caspase-1 in their keratinocytes (KIL-18Tg and KCASP1Tg, respectively) spontaneously produce IgE in an endogenous IL-18–dependent manner (10, 15, 16). Taken together, these results suggest that IL-18 promotes allergic disorders, particularly intrinsic type atopic diseases characterized by the absence of sensitivity to a particular antigen.

Although it is well established that IL-18 stimulates CD4 + T cells to produce IL-4 in vivo and in vitro, the CD4 + T cell subset that responds to IL-18 in vivo by inducing IL-4 and CD40L expression remains unknown. NKT cell subset that responds to IL-18 in vivo by inducing IL-4 activity to a particular antigen.

Materials and Methods
Mice and Reagents. Specific pathogen-free female BALB/c and C57BL/6 mice, and C57BL/6 class II−/− mice at 8 wk of age were purchased from The Jackson Laboratory. C57BL/6 IL-4−/− mice were obtained from Taconic Farms. Mouse Tg for β2 TCR recognizing OVA233–339 (DO11.10; BALB/c genetic background) were provided by D. Loh (Washington University, St. Louis, MO). The generation of C57BL/6 CD1d−/− mice was previously described (23). Recombinant mouse IL-18 and anti–mouse IL-18Rα chain mAb (Y38; reference 24) were provided by Hayashibara Biochemical Laboratories Inc. FITC–anti-mouse CD4 (GK1.5), FITC–anti–mouse CD44 (IM7), FITC–anti–rat IgG1 (RG11/39.4), FITC–anti–mouse IL-4 (BVD4–ID11), CyChrome–anti–mouse CD4 (RM4–5), biotinylated anti–mouse CD40L, PE–anti–mouse NK1.1 (PK136), and PE-labeled streptavidin were purchased from BD Biosciences.

Preparation of NKT Cells. Splenic CD4 + T cells from C57BL/6 mice were purified by MicroBeads (anti–mouse CD4, clone RM4–5; Miltenyi Biotec). The enriched CD4 + T cells were first treated with 10 μg/ml anti-FcγRII/III for 30 min at 4°C followed by treatment with FITC–anti–CD4 and PE–anti–NK1.1 for 30 min at 4°C in staining buffer (PBS, 1% FCS). Both CD4 + NK1.1 + and CD4 + NK1.1 − T cells were sorted by using a fluorescence cell sorter (Elite; Coulter Electronics). Purity of each population was >98.5% after reanalysis. In some experiments, CD4 + NK1.1 + T cells were negatively enriched by depletion of other cell populations from spleen cells. Cell depletion was performed by treatment with a mixture of FITC–anti–B220, FITC–anti–CD8, FITC–anti–I-Ak, FITC–anti–NK1.1, and FITC–anti–FcyRII/III antibodies (BD Biosciences) and subsequent incubation with anti–FITC-coated magnetic beads (Miltenyi Biotec), followed by two rounds of exposure to a magnetic field. The enriched CD4 + NK1.1 + T cells were stained with FITC–anti–CD44 and PE–anti–NK1.1, and CD4 + NK1.1 − T cells were sorted.

In Vitro Culture. Sorted CD4 + NK1.1 + and CD4 + NK1.1 − T cells from C57BL/6 mice (10 5/0.2 ml/well) were cultured with medium alone or various combinations of 200 mM IL-2, 10 ng/ml IL-12, and 50 ng/ml IL-18 for 4 d in RPMI 1640 supplemented with 10% FBS, 50 μM 2-ME, 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. Supernatants were harvested and tested for IL-4, IL-9, IL-13, and IFN-γ contents by ELISA. The collected T cells were also examined for their expression of CD40L and capacity to induce B cells to produce IgE by incubation with highly purified B cells as previously described (10).

In Vivo Treatment of Mice. Mice were injected on a daily basis with PBS buffer or IL-18 (2 μg/day) for 13 d. For adoptive transfer experiments, class II−/− mice were transferred with 10 × 10 5 purified CD4 + NK1.1 − T cells from either WT or IL-4−/− mice intravenously. From the day after cell transfer, mice were treated daily with 2 μg IL-18 as described above. They were bled 0, 7, 10, and 14 d later and serum IgE, IL-4, and IL-13 were measured by ELISA. In some experiments, CD4 + NK1.1 + T cells from WT mice were labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE; Molecular Probes, Inc.; reference 25) and transferred to class II−/− mice. Cells containing transferred cells were stained with PE–anti–CD4 and transferred cells were identified by their CFSE fluorescence and CD4 expression. The frequency of repopulated CD4 + T cells in class II−/− mice was calculated by (number of CFSE + CD4 + cells) / (number of transferred cells).

Flow Cytometry. To detect IL-4–producing cells from mice treated with IL-18, total spleen cells derived from C57BL/6 mice that had been injected with IL-18 for 10 d were first stained with CyChrome–anti–CD4 and PE–anti–NK1.1 and followed by fixa-
tion with 4% (wt/vol) paraformaldehyde in PBS and permeabilization of cell membrane with ice-cold PBS containing 1% FCS plus 0.1% saponin. Resultant cells were further stained with 0.5 g FITC-anti–mouse IL-4 or isotype-matched control Ab and analyzed for their proportion of cytoplasmic IL-4 cells by FACS-Calibur® (Becton Dickinson). To detect CD40L+ cells in mice treated with IL-18, total spleen cells were stained with FITC–anti-CD4, PE–anti-NK1.1, and the combination of biotinylated anti-CD40L and tri-color streptavidin (Caltag), and then analyzed on a FACS-Calibur®.

For determination of IL-18Rα chain expression on NKT cells, after FcR blocking with anti-FcγRI/II, splenic lymphocytes were incubated with anti–mouse IL-18Rα chain mAb or control rat IgG1 mAb (R3-34) for 30 min at 4°C, followed by FITC-conjugated anti-rat IgG1 mAb, PE–anti-NK1.1, and Cy-Chrome–anti–mouse CD4 for 30 min at 4°C in staining buffer. Samples were analyzed on a FACS-Calibur®.

Results

**CD1−/− Mice Are Defective in the Production of Th2 Cytokines and IgE in Response to IL-18 Administration.** IL-18 treatment of BALB/c mice induces IgE in a CD4+ T cell–dependent manner (10). Consistent with our previous report (10), this IgE response is not associated with induction of Th2 cells (not depicted), suggesting that TCR engagement might not be required for IgE induction in IL-18–injected mice. To further substantiate this observation that IgE response is independent of TCR engagement by endogenous Ags, we examined the capacity of BALB/c mice expressing transgene–encoding TCR for OVA peptide (DO11.10 mice) to produce IgE in response to IL-18. These mice received daily injections of IL-18 (2 μg/day) for 13 d. Like normal BALB/c mice, they produced IgE in response to this treatment (Fig. 1 A), although this IL-18 treatment again did not induce Th2 response (not depicted). Furthermore, this IL-18–induced IgE production was entirely resistant to cyclosporin A treatment (not depicted), further excluding the involvement of TCR-mediated T cell activation in this T cell–dependent IgE response. To identify the IL-18–responsive T cells that are relevant in IL-18–induced IgE response, we compared the capacity of C57BL/6 and C57BL/6 background CD1−/− mice lacking CD4+ NK1.1+ T cells (23) to produce IgE in response to IL-18. As shown in Fig. 1 B, IL-18 caused a striking increase in serum IgE levels in WT mice but not in CD1−/− mice. Furthermore, administering IL-18 to WT mice caused the production of a significant amount of IL-4 and IL-13 whereas CD1−/− mice produced no IL-4 and diminished amounts of IL-13 (Fig. 1, C and D). These results suggest that CD4+ NK1.1+ T cells produce both IL-4 and IL-13 in response to IL-18.

NKT Cells Produce IL-4 and Express CD40L in Response to In Vivo Treatment with IL-18. To determine the roles of NKT cells in IL-18–induced IgE response, we directly tested whether CD4+ NK1.1+ T cells produce IL-4 and increase CD40L expression when WT mice are injected with IL-18. As shown in Fig. 2 A, CD4+ NK1.1+ T cells obtained from IL-18–injected mice, compared with PBS-injected mice, showed a significant increase (P < 0.01) in the proportion of T cells producing IL-4 ex vivo (7.1%). In contrast, few, if any, CD4+ NK1.1− T cells contained cyto-

![Figure 1](https://example.com/figure1.png)

**Figure 1.** CD1−/− mice fail to produce IgE and IL-4 in response to in vivo treatment with IL-18. (A–D) BALB/c, BALB/c background DO11.10 Tg, C57BL/6, and C57BL/6 background CD1−/− mice (five mice per group) were injected daily with IL-18 (2 μg/day) for 13 d. Mice were bled on days 0, 7, 10, and 14 and serum IgE (A and B), IL-4 (C), and IL-13 (D) were measured by ELISA. Results are geometric means ± SD. *, <10 pg/ml.
plasmic IL-4. Similarly, CD4+ NK1.1+ T cells in IL-18–injected WT mice showed a significant increase (P < 0.01) in the proportion of CD40L-expressing T cells (Fig. 2 B). Although IL-18 also caused an increase in the proportion of CD4+ NK1.1+ T cells that expressed CD40L, the frequency of positive cells was significantly less than that among the CD4+ NK1.1+ T cells.

IL-18α Chain Expression on NKT Cells. The responsiveness of CD4+ NK1.1+ T cells to IL-18 suggested that these cells express IL-18R constitutively. We compared the expression of IL-18α chain on CD4+ NK1.1+ and CD4+ NK1.1− T cells by flow cytometry using anti-IL-18α chain mAb. NK cells (CD4− NK1.1+), which express high levels of IL-18α chain (24), were included as positive controls. Freshly prepared splenic CD4+ NK1.1+ T cells (Fig. 2 C, R2) constitutively express high levels of IL-18α chain (mean fluorescence intensity [MFI]: 11.0) whereas CD4+ NK1.1− T cells (Fig. 2 C, R1) express only low levels (MFI: 3.9). Consistent with previous reports of ours (24) and others (11), NK cells (Fig. 2 C, R3) constitutively express high levels of IL-18α chain. They produce IL-13 but not IL-4 upon IL-18 stimulation in vitro (not depicted).

IL-18 Stimulates NKT Cells to Produce IL-4, Express CD40L, and Enhance IgE Production by B Cells. To further substantiate the capacity of CD4+ NK1.1+ and CD4+ NK1.1− T cells to produce IL-4 and express CD40L in response to IL-18, we sorted CD4+ NK1.1+ and CD4+ NK1.1− T cells and stimulated them with 200 pM IL-2 and/or 50 ng/ml IL-18 for 4 d. As shown in Fig. 3 A, CD4+ NK1.1+ T cells cultured with IL-18 and IL-2 produced significant amounts of IL-4 and IL-13 whereas conventional CD4+ NK1.1− T cells produced little IL-4 and small amounts of IL-13. We also found that IL-18 plus IL-2 stimulates CD4+ NK1.1+ but not CD4+ NK1.1− T cells to produce IL-9 (Fig. 3 A). CD4+ NK1.1+ T cells cultured with IL-18 and IL-2 produced modest amounts of IFN-γ (249 pg/ml), far less than that (5,381 pg/ml) stimulated by IL-18 and IL-12 (not depicted).

As shown in Fig. 3 B, IL-2 or IL-18 alone substantially increased CD40L expression on CD4+ NK1.1+ T cells (46.1 and 39.7%), but the combination caused profound induction (94.2%). In contrast, the same treatment of CD4+ NK1.1− T cells only moderately induced CD40L, suggesting that CD4+ NK1.1+ T cells are the major targets of IL-18.

We also tested the capacity of CD4+ NK1.1+ T cells and CD4+ NK1.1− T cells stimulated with IL-2 and IL-18 for 4 d to induce IgE in resting B cells in vitro. IL-2 plus IL-18–stimulated CD4+ NK1.1+ T cells were able to induce B cells to secrete IgE whereas conventional CD4+ NK1.1− T cells failed (Table I). Taken together, these results indicate that NKT cells constitutively expressing IL-18α chain are a crucial subset of CD4+ T cells that respond to IL-18 by producing IL-4 and expressing CD40L, which in combination, induce IgE production by B cells both in vivo and in vitro.

NKT Cells but Not Previously Activated CD4+ NK1.1− T Cells Produce IL-4 in Response to IL-18. In Fig. 3, we demonstrated that NKT cells but not CD4+ NK1.1− T cells are highly responsive to IL-18. To determine if the induction of Th2 cytokines and CD40L after IL-18 is a unique property of NKT cells or a property of all previously activated or memory T cells, we compared IL-18 responsiveness of NKT cells to that of previously activated...
conventional CD4+ T cells. For this purpose, we used CD44high CD4+ T cells as a control population. Before comparison, we examined the expression of CD44 on total CD4+ T cells, CD4+ NK1.1+ T cells, and CD4+ NK1.1− T cells obtained from normal C57BL/6 mice. As shown in Fig. 4 A, almost all freshly prepared CD4+ NK1.1+ T cells (R2) expressed high levels of CD44 whereas only 12.9 and 21.6% of CD4+ NK1.1− T cells (R3) and total CD4+ T cells (R1) expressed CD44, respectively.

Because a substantial proportion of CD44high CD4+ T express NK1.1.1 T cells, CD4+ NK1.1+ T cells, and CD4+ NK1.1− T cells obtained from normal C57BL/6 mice. As shown in Fig. 4 A, almost all freshly prepared CD4+ NK1.1+ T cells (R2) expressed high levels of CD44 whereas only 12.9 and 21.6% of CD4+ NK1.1− T cells (R3) and total CD4+ T cells (R1) expressed CD44, respectively.

Because a substantial proportion of CD44high CD4+ T express NK1.1.1 T cells, we compared IL-18 responsiveness of NKT cells to that of CD44high NK1.1− CD4+ T cells or CD44low NK1.1− CD4+ T cells. As NKT cells sometimes lose their NK1.1 expression during or after stimulation (26), we sorted CD44high NK1.1− CD4+ T cells from splenic CD4+ T cells, already depleted of other cell populations, particularly NKT cells (refer to Materials and Methods). We also sorted CD44low NK1.1− T cells and CD44high NK1.1+ CD4+ T cells (NKT cells) from total CD4+ T cells. We stimulated these three populations with IL-2 and/or IL-18. Consistent with the results shown in Fig. 3 A, only NKT cells produced both IL-4 and IL-13 in response to IL-2 plus IL-18 (Fig. 4 C). These results support our conclusion that NKT cells but not previously activated conventional T cells have the capacity to induce IgE from B cells.

**Table I.** Induction of IgE Production by B Cells Cocultured with IL-18 and IL-2–stimulated NKT Cells or Conventional CD4+ T Cells

<table>
<thead>
<tr>
<th>Cells</th>
<th>Culture conditions</th>
<th>IgE (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+ NK1.1− T cells</td>
<td>medium</td>
<td>&lt;10</td>
</tr>
<tr>
<td>CD4+ NK1.1− T cells</td>
<td>IL-2 plus IL-18</td>
<td>&lt;10</td>
</tr>
<tr>
<td>CD4+ NK1.1+ T cells</td>
<td>medium</td>
<td>&lt;10</td>
</tr>
<tr>
<td>CD4+ NK1.1+ T cells</td>
<td>IL-2 plus IL-18</td>
<td>90.1 ± 3.7</td>
</tr>
</tbody>
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CD4+ NK1.1+ and CD4+ NK1.1− T cells (10⁵/0.2 ml/well) from C57BL/6 mice were cultured with medium alone or 50 ng/ml IL-18 and 200 pM IL-2. After 4 d of culture, freshly purified C57BL/6 splenic B cells (10⁵/0.2 ml/well) were added with 10 μg/ml anti–IFN-γ antibody. After an additional 10 d of incubation, supernatants were harvested and IgE contents were measured by ELISA. Results are geometric means ± SD.
CD4⁺ NK1.1⁻ T Cells Are Required in IL-18–induced IgE Responses. Although conventional CD4⁺ NK1.1⁻ T cells responded poorly to IL-18 in vivo and in vitro with relatively modest induction of IL-4 and CD40L, these results leave open the question of whether conventional CD4⁺ T cells are also required for the observed effect of IL-18 on IgE production. Thus, we tested IL-18 responsiveness of class II⁻/⁻ mice expressing almost the same number of CD4⁺ NK1.1⁻ T cells as were expressed by WT mice, although CD4⁺ T cells constituted only 4 to 5% of their spleen cells (Fig. 5 A; reference 20). As shown in Fig. 5 B, class II⁻/⁻ mice completely failed to demonstrate induction of IgE in response to IL-18 treatment. However, class II⁻/⁻ mice reconstituted with conventional CD4⁺ T cells from WT mice mounted a small but significant IgE response to IL-18 (Fig. 5 C) whereas those mice reconstituted with conventional CD4⁺ T cells from IL-4⁻/⁻ mice failed to do so in response to IL-18 (Fig. 5 C). Compared with WT mice, class II⁻/⁻ mice reconstituted with CD4⁺ T cells showed weak IgE responses, suggesting that host spleen was only partially repopulated. Indeed, only 0.94, 1.58, and 0.64% of transferred conventional T cells were repopulated at days 3, 7, and 10, respectively (Fig. 5 D). These results provide direct evidence that IL-4–producing conventional CD4⁺ T cells are needed for IgE production by B cells.

Discussion

Here we show that the administration of IL-18 results in increases in serum levels of IgE, IL-4, and IL-13 in normal mice but not in CD1⁻/⁻ mice, which lack NKT cells (Fig. 1). In addition, NKT cells, which are strongly positive for the IL-18Rα chain, produce IL-4, IL-9, and IL-13 and express CD40L in response to IL-18 plus IL-2 in the absence of TCR engagement (Figs. 2 and 3). Furthermore, NKT cells that are stimulated with IL-18 and IL-2 for 4 d promote class
switching to IgE in B cells in vitro (Table I). However, class II−/− mice, which have NKT cells but lack conventional T cells, fail to produce IgE in response to IL-18 treatment, suggesting the importance of conventional T cells in IL-18–induced IgE response (Fig. 5). Indeed, these mice were able to produce IgE after reconstitution with conventional T cells from WT but not from IL-4−/− mice. Taken together, these results demonstrate that NKT cells are relevant cells for IL-18–induced IL-4 production and suggest that they recruit the action of conventional T cells in the induction of IgE.

In atopic individuals, it is well known that allergens give rise to a polarization to Th2 responses and that enhanced secretion of IL-4 promotes IgE production (27). Allergen binding to IgE cross-links FcεR on basophils and mast cells, which causes them to produce IL-3, IL-4, IL-5, IL-9, IL-13, and a variety of chemical mediators, most notably histamine (28). The combination of these products induces allergic inflammation, highlighting the importance of IgE for activating basophils and mast cells. Thus, we could designate this IgE–dependent allergic disease as “acquired type allergic response.” However, we have recently demonstrated an alternative, IgE-independent activation pathway of mast cell/basophil (“innate type allergic response”; reference 16). IL-18 in combination with IL-3 directly stimulates these cells to produce IL-4, IL-13, and histamine in an IgE-independent manner (9). IL-18 also stimulates CD4+ T cells to produce IL-4 and express CD40L, which can induce in vitro class switching to IgE in B cells in an Ag-independent manner (10). Our previous study also demonstrated that IL-18–treated mice or IL-18–producing caspase-1 Tg mice express high serum levels of IgE, which is CD4+ T cell−, IL-4−, and signal transducer and activator of transcription 6–dependent, but Th2 cell independent (10). Here we show that IL-18 stimulates IgE production in OVA-specific TCR Tg mice without OVA administration (Fig. 1 A). Furthermore, we found that this IgE response is Ag nonspecific and resistant to cyclosporin A treatment. However, IL-18 is not necessarily essential for induction of IgE response because like WT mice, IL-18–deficient mice generate a Th2 response and produce IgE when inoculated with the helmint Nippostrongylus brasiliensis (unpublished data). Thus, N. brasiliensis–induced Th2 responses, which require TCR engagement, can be generated in the absence of IL-18. These results indicate that IgE response to antigen using the TCR is possibly not affected by blocking IL-18 as a therapeutic strategy but is susceptible to the treatment with cyclosporin A. In other words, IL-18–induced IgE responses become apparent only in the absence of TCR engagement. Thus, IL-18 may induce allergic disorders, particularly intrinsic atopic diseases characterized by the absence of particular allergen–specific IgE. Our results suggest that it is important to determine whether observed IgE response is dependent on TCR and/or IL-18–mediated signaling.

The novel lymphoid lineage, NKT cells, which expresses both NK receptors and TCR encoded by the Vα14 and Jα281 gene segments, has been suggested to play an important role in the regulation of immune responses (17). Cytokines such as IL-12 can stimulate NKT cells to release IFN-γ and exhibit natural cytotoxicity (29, 30). Recent studies demonstrated that NKT cells could transactivate NK cells via IFN-γ production upon stimulation with CD1d-bound glycolipid L (α-GalCer; reference 18). This IFN-γ production by NKT cells in response to α-GalCer is predominantly mediated by IL-12 produced by dendritic cells (31). Moreover, α-GalCer–stimulated NKT cells inhibit antigen–induced allergic responses by production of IFN-γ

Figure 5. Transfer of conventional CD4+ T cells from WT mice partially reconstitutes IgE production by class II−/− mice injected with IL-18. (A) Frequency of CD4+ NK1.1+ T cells in total spleen cells (top) and CD4–enriched spleen cells (bottom) from C57BL/6, CD1−/−, and class II−/− mice. Percent of cells in selected quadrants are indicated. C57BL/6 and class II−/− mice received either nothing (B) or 10^7 CD4+ NK1.1+ T cells from WT or IL-4−/− mice (C; five mice per group) and were injected daily with IL-18 (2 µg/day) for 13 d. Mice were bled on days 0, 7, 10, and 14 and serum IgE was measured by ELISA. (D) Class II−/− mice (three mice per group) received an intravenous injection of 10^7 CFSE–labeled CD4+ T cells from C57BL/6 mice. Spleen cells were isolated from recipients at various times after transfer and transferred cells were identified by CSFE fluorescence as described in Materials and Methods.
Production by both NKT and conventional CD4 T cells is critical for IL-18–induced IgE production. However, as NKT cells and conventional T cells produce large and small amounts of IL-4, respectively, the precise role of IL-4 from NKT cells and conventional T cells in B cell activation remains uncertain. Furthermore, we do not know the appropriate ratio of NKT cells to conventional T cells in effective B cell IgE responses.

We have shown that KIL-18Tg and KCASP1Tg mice, which overexpress mature IL-18 or caspase-1 gene, respectively, in keratinocytes, spontaneously produce IgE in an endogenous IL-18–dependent manner (10, 15, 16). Interestingly, our recent examination of KCASP1Tg mice revealed that they exhibited a two- to threefold increase in splenic NKT cells, although short-term in vivo treatment with IL-18 did not affect the number of splenic NKT cells (unpublished data). These increases in the proportion of splenic NKT cells and serum IgE levels in KCASP1Tg mice are somewhat similar to that in the Vα14-Jα281 Tg mice established by Bendelac et al. (33). These Vα14-Jα281 Tg mice exhibit a selective increase in serum IgE (sixfold above controls on average) and IgG1 (twofold above controls). Here, we have provided strong evidence that CD1d-restricted NKT cells are critically important for the induction of IgE in response to IL-18. However, as NKT cells that express TCR other than Vα14-Jα281 TCR are also selected by CD1d (34), it remains possible that such nonclassical NKT cells also contribute to the IL-18–induced IgE response. Future studies with Jα281−/− mice should be able to resolve this issue.

Recently, Leite-de-Maraes et al. (35) have reported that IL-18 enhances IL-4 production by L-activated NKT cells but not by conventional T cells. Thus, IL-18 enhances anti-CD3 Ab– or α-GalCer–induced IL-4 production by NKT cells. However, IL-18 also enhanced IFN-γ production by NKT cells stimulated with anti-CD3 or α-GalCer. These results suggest that IL-18 exerts its action on IL-4 production by NKT cells by amplifying the signaling pathway initiated by TCR/CD3 cross-linkage or cognate L-α-GalCer. In sharp contrast, our results reveal that IL-18 and IL-2 synergistically and directly exert IL-4 and CD40L–inducing activities on NKT cells even in the absence of TCR engagement. Furthermore, these activated NKT cells in collaboration with IL-4–producing conventional T cells can induce class switching to IgG1 in B cells, indicating a new function of NKT cells in innate immunity. These findings suggest that IL-18 and NKT cells might be potential targets in the effort to develop agents that regulate Th2-independent allergic disorders as innate type allergic response. Furthermore, as IL-18 predominantly enhances production of Th2 cytokines both in vitro and in vivo, injection of IL-18 might be promising for the treatment of Th1 diseases such as insulin dependent diabetes mellitus.

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