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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 Nakanishi[1,2]

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

Address correspondence to Kenji Nakanishi, Department of Immunology and Medical Zoology, Hyogo College of Medicine, 1-1 Mukogawa-cho, Nishinomiya, Hyogo 663-8501, Japan. Phone: 81-798-45-6572; Fax: 81-798-40-5423; E-mail: nakaken@hyo-med.ac.jp

**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 production and CD40L expression remains unknown. NKT cells express the NK cell marker NK1.1 and an invariant TCR Vα14-Jα281 chain, preferentially associated with a Vβ8.2 chain (17). NKT cells are positively selected by the nonpolymorphic MHC class I–like molecule CD1d (17) and recognize glycolipids, such as α galactosylceramide (α-GalCer) presented by CD1d (18). ~60% of all NKT cells express CD4 whereas the remaining cells are $CD4^-$ $CD8^-$ (17). NKT cells exert regulatory functions, which are most likely mediated by their capacity to promptly release large amounts of IL-4 and IFN-γ upon TCR engagement by anti-CD3 or NKT cell Ls such as α-GalCer (19–22). Modulation of NKT cells may not only determine the outcome of host immune response, but also be applicable for the treatment of immunological diseases. Furthermore, it is important to determine the stimulus that selectively induces NKT cells to produce Th1 or Th2 cytokines.

Here we demonstrate that IL-18 treatment of mice induces CD40L expression and IL-4 production by NKT cells in vivo. NKT cell–deficient (CD1d-deficient; $CD1d^{-/-}$) mice or conventional $CD4^+$ T cell–deficient (class II–deficient; class $II^{-/-}$) mice fail to produce IgE in response to injection of IL-18 whereas class $II^{-/-}$ mice reconstituted with conventional $CD4^+$ T cells produce a substantial amount of IgE. Culturing NKT cells with IL-2 and IL-18, without TCR engagement, causes a striking increase in CD40L expression and production of significant amounts of IL-4, IL-9, and IL-13 by these cells. Although conventional $CD4^+$ $NK1.1^-$ T cells respond poorly to IL-18 with relatively modest induction of IL-4 and CD40L, they are required for IgE production in response to IL-18 in vivo, as shown by the failure of class $II^{-/-}$ mice reconstituted with conventional $CD4^+$ T cells from $IL\text{-}4^{-/-}$ mice to produce IgE when treated with IL-18. These results indicate that NKT cells are a critical subset of $CD4^+$ T cells that respond to IL-18 by expression of Th2 cytokines and CD40L in vivo, and conventional $CD4^+$ T cells act as Th cells together with NKT cells in IL-18–induced IgE responses.

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 $IL\text{-}4^{-/-}$ mice were obtained from Taconic Farms. Mice Tg for αβ TCR recognizing $OVA_{323–339}$ (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 (BVD4–1D11), 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–$I\text{-}A^d$, FITC–anti-NK1.1, and FITC–anti-FcγRII/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 $CD44^{high}$ $NK1.1^-$ 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 pM 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 10×10^6 purified $CD4^+$ $NK1.1^-$ T cells from either WT or $IL\text{-}4^{-/-}$ 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 μg FITC-anti–mouse IL-4 or isotype-matched control Ab and analyzed for their proportion of cytoplasmic IL-4$^+$ cells by FACSCalibur® (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 FACSCalibur®.

For determination of IL-18Rα chain expression on NKT cells, after FcR blocking with anti-FcγRII/III, 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 FACSCalibur®.

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-

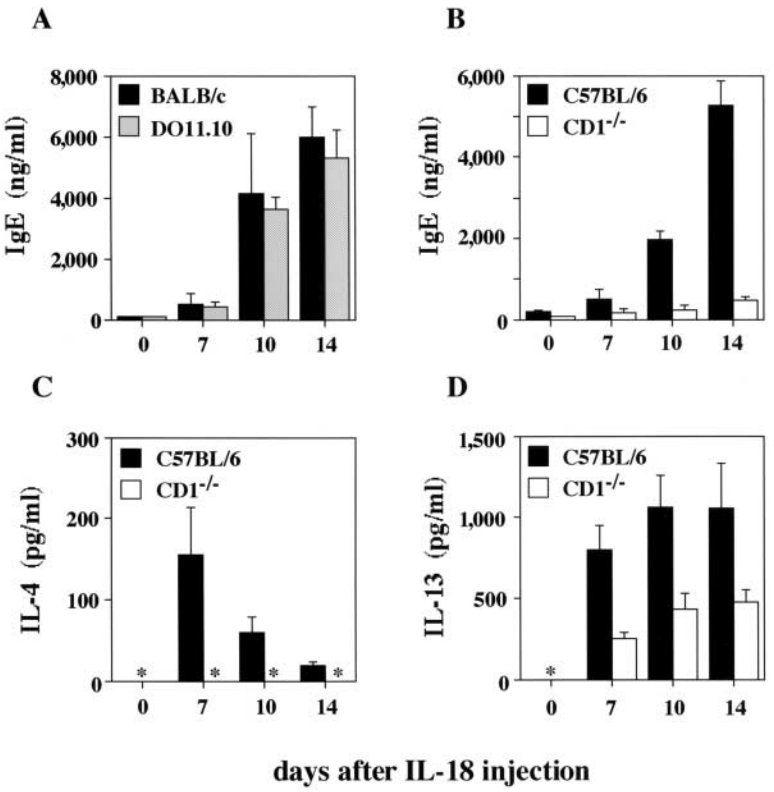

Figure 1. CD1$^{-/-}$ mice fail to produce IgE and IL-4 in response to in vivo treatment with IL-18. (A–D) BALB/c, BALB/c background DO11.10 Tg, C57BL/6, and C57BL/6 background CD1$^{-/-}$ mice (five mice per group) were injected daily with IL-18 (2 μg/day) for 13 d. Mice were bled on days 0, 7, 10, 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.

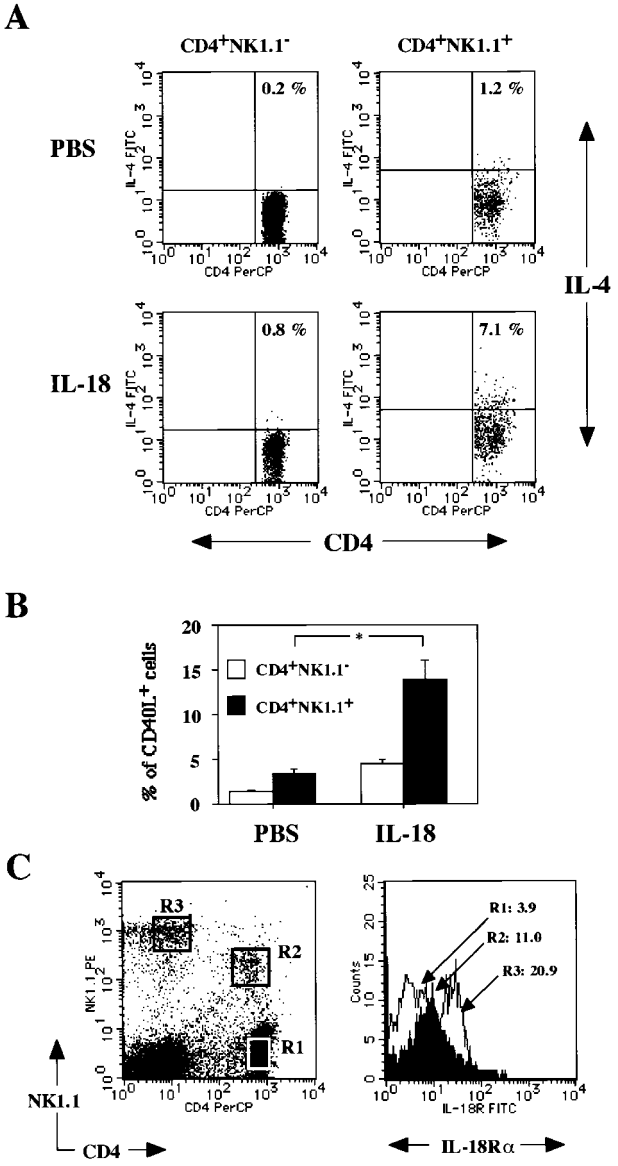

Figure 2. NKT cells produce IL-4 and express CD40L in response to in vivo treatment with IL-18. C57BL/6 mice were injected daily with IL-18 (2 μg/day) or PBS for 10 d (four mice per group). Intracellular IL-4 (A) or CD40L staining (B) of $CD4^+$ $NK1.1^+$ and $CD4^+$ $NK1.1^-$ T cells was performed as described in Materials and Methods. Representative data are shown from four independent mice. The percentage shown represents the proportion of cells positive for cytoplasmic IL-4. Results are geometric means ± SD. *, $P < 0.01$. (C) Freshly prepared splenic cells from C57BL/6 mice were analyzed for expression of CD4 and NK1.1 by flow cytometry. IL-18Rα chain expression on $CD4^+$ $NK1.1^-$ (R1), $CD4^+$ $NK1.1^+$ (R2), and $CD4^-$ $NK1.1^{high}$ (R3) cells was analyzed as described in Materials and Methods. The number shown represents the MFI of IL-18Rα chain staining.

IL-18Rα 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-18Rα chain on $CD4^+$ $NK1.1^+$ and $CD4^+$ $NK1.1^-$ T cells by flow cytometric analysis using anti–IL-18Rα chain mAb. NK cells ($CD4^-$ $NK1.1^{high}$), which express high levels of IL-18Rα 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-18Rα 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-18Rα 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-18Rα 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

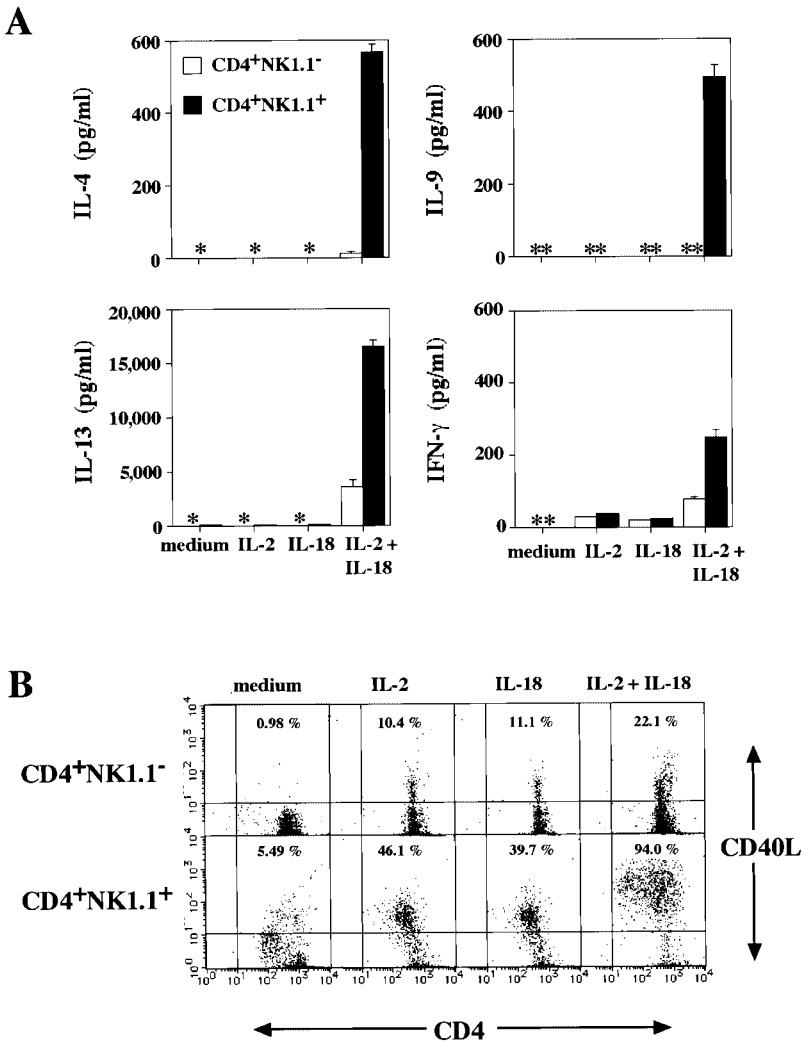

Figure 3. NKT cells produce IL-4, IL-9, and IL-13 and express CD40L in vitro in response to IL-18. Sorted $CD4^+$ $NK1.1^+$ and $CD4^+$ $NK1.1^-$ T cells (10^5/0.2 ml/well) from C57BL/6 mice were cultured with various combinations of 200 pM IL-2 and 50 ng/ml IL-18. After 4 d of culture, supernatants were harvested and tested for IL-4, IL-9, IL-13, and IFN-γ contents by ELISA (A) and surface expression of CD40L was analyzed by flow cytometry (B). The percentage shown represents the proportion of $CD40L^+$ cells among $CD4^+$ T cells. Results are geometric means ± SD. *, <10 pg/ml; **, <40 pg/ml.

conventional $CD4^+$ T cells. For this purpose, we used $CD44^{high}$ $CD4^+$ 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.

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

Cells	Culture conditions	IgE (ng/ml)
$CD4^+$ $NK1.1^-$ T cells	medium	<10
	IL-2 plus IL-18	<10
$CD4^+$ $NK1.1^+$ T cells	medium	<10
	IL-2 plus IL-18	90.1 ± 3.7

$CD4^+$ $NK1.1^+$ and $CD4^+$ $NK1.1^-$ T cells (10^5/0.2 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 (10^5/0.2 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.

Because a substantial proportion of $CD44^{high}$ $CD4^+$ T express NK1.1 T cells, we compared IL-18 responsiveness of NKT cells to that of $CD44^{high}$ $NK1.1^-$ $CD4^+$ T cells or $CD44^{int}$ $NK1.1^-$ $CD4^+$ T cells. As NKT cells sometimes lose their NK1.1 expression during or after stimulation (26), we sorted $CD44^{high}$ $NK1.1^-$ $CD4^+$ T cells from splenic $CD4^+$ T cells, already depleted of other cell populations, particularly NKT cells (refer to Materials and Methods). We also sorted $CD44^{int}$ $NK1.1^-$ T cells and $CD44^{high}$ $NK1.1^+$ $CD4^+$ 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.

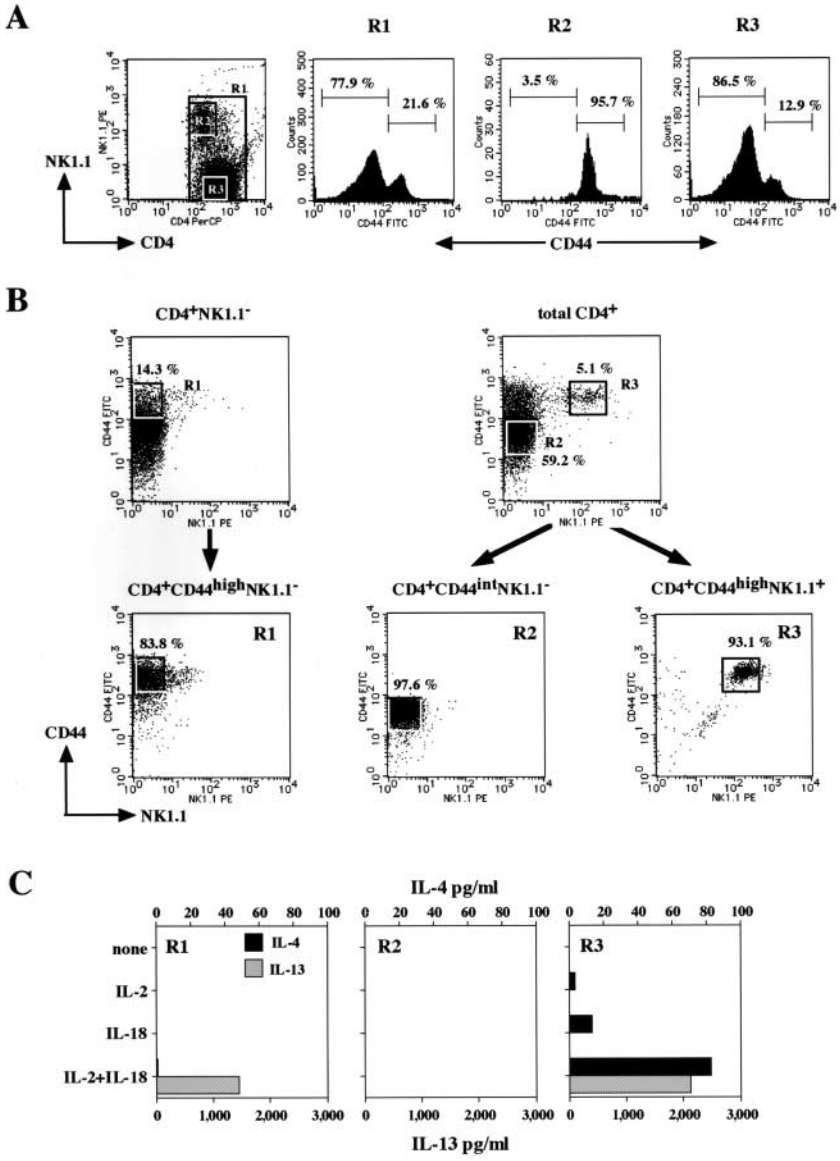

Figure 4. Previously activated $CD4^+$ $NK1.1^-$ T cells fail to produce IL-4 in response to IL-18. (A) Freshly prepared $CD4^+$ cells from C57BL/6 mice were analyzed for expression of CD44 gated on total CD4 (R1), $CD4^+$ $NK1.1^+$ (R2), and $CD4^+$ $NK1.1^-$ (R3) T cells by flow cytometry. (B) Total $CD4^+$ T cells were negatively depleted of NKT cells as well as other cell populations and then positively sorted for $CD44^{high}$ $NK1.1^-$ T cells (R1). $CD44^{int}$ $NK1.1^-$ T cells (R2) and NKT cells (R3) were positively sorted from total $CD4^+$ T cells. (C) Sorted $CD4^+$ $CD44^{high}$ $NK1.1^-$, NKT, and $CD4^+$ $CD44^{int}$ $NK1.1^-$ T cells (10^5/0.2 ml/well) were cultured with various combinations of 200 pM IL-2 and 50 ng/ml IL-18. After 4 d of culture, supernatants were harvested and tested for IL-4 and IL-13 by ELISA.

$CD4^+$ $NK1.1^-$ 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 $IL\text{-}4^{-/-}$ 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

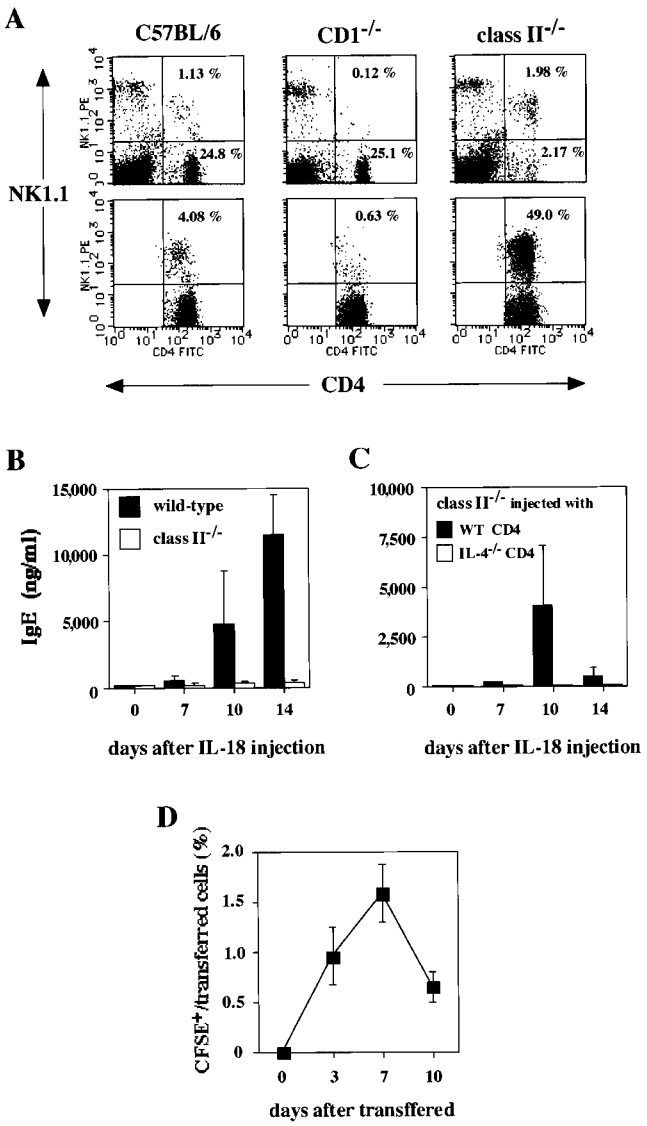

Figure 5. Transfer of conventional $CD4^+$ T cells from WT mice partially reconstitutes IgE production by class $II^{-/-}$ mice injected with IL-18. (A) Frequency of $CD4^+$ $NK1.1^+$ T cells in total spleen cells (top) and CD4-enriched spleen cells (bottom) from C57BL/6, $CD1^{-/-}$, and class $II^{-/-}$ 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 $IL\text{-}4^{-/-}$ 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.

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 $IL\text{-}4^{-/-}$ 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 helminth *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 importance 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 cytotoxity (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

(32). However, NKT cells also exert regulatory functions, most likely through their capacity to promptly release large amounts of IL-4 upon stimulation with anti-CD3 or α-GalCer, thus promoting the acquisition of a Th2 phenotype (19–22). Therefore, NKT cells can promote Th1 responses in certain situations and Th2 responses in others.

The data presented here show that among $CD4^+$ T cells, NKT cells have the unique capacity to respond to IL-18 by IL-4 production and CD40L up-regulation and directly help B cells produce IgE in vitro and in vivo without antigenic stimulation. However, our studies with class $II^{-/-}$ mice reveal that conventional $CD4^+$ T cells are also required for the observed effect of IL-18 on IgE production (Fig. 5). Class $II^{-/-}$ mice reconstituted with conventional $CD4^+$ T cells from WT mice but not from $IL\text{-}4^{-/-}$ mice mounted a small but significant IgE response to IL-18 (Fig. 5 C), suggesting that conventional T cells participate in IgE induction by secreting a small amount of IL-4. However, this IgE production in WT $CD4^+$ T cell reconstituted mice failed to increase beyond day 10 after IL-18 administration. We suspect that the failure to completely reconstitute IL-18–induced IgE responses in these animals is due to incomplete repopulation of $CD4^+$ T cells in class $II^{-/-}$ hosts (Fig. 5 D). We could increase the number of repopulated cells by increasing the number of transferred cells, although we could not increase repopulation rate (unpublished data). Thus, in the absence of class II expression, $CD4^+$ T cells may not survive efficiently and are unable to completely reconstitute IgE production. Nevertheless, our finding indicates that IL-4 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 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 $J\alpha281^{-/-}$ 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 IgE 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 insulin dependent diabetes mellitus.

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