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# Contribution of IL-18 to Th1 Response and Host Defense Against Infection by *Mycobacterium tuberculosis*: A Comparative Study with IL-12p40

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**The present study was conducted to critically determine the protective role of IL-18 in host response to *Mycobacterium tuberculosis* infection. IL-18-deficient (knockout (KO)) mice were slightly more prone to this infection than wild-type (WT) mice. Sensitivity of IL-12p40KO mice was lower than that of IL-12p40/IL-18 double KO mice. IFN-γ production caused by the infection was significantly attenuated in IL-18KO mice compared with WT mice, as indicated by reduction in the levels of this cytokine in sera, spleen, lung, and liver, and its synthesis by spleen cells restimulated with purified protein derivatives. Serum IL-12p40 level postinfection and its production by peritoneal exudate cells stimulated with live bacilli were also significantly lower in IL-18KO mice than WT mice, suggesting that attenuated production of IFN-γ was secondary to reduction of IL-12 synthesis. However, this was not likely the case, because administration of excess IL-12 did not restore the reduced IFN-γ production in IL-18KO mice. In further studies, IL-18 transgenic mice were more resistant to the infection than control littermate mice, and serum IFN-γ level and its production by restimulated spleen cells were increased in the former mice. Taken together, our results indicate that IL-18 plays an important role in Th1 response and host defense against *M. tuberculosis* infection although the contribution was not as profound as that of IL-12p40. *The Journal of Immunology*, 2002, 169: 323–329.**

Tuberculosis is a reemerging infectious disease, which has recently attracted the attention of many investigators due to its increased morbidity and mortality rate and appearance of multidrug resistant *Mycobacterium tuberculosis* that resists conventional chemotherapy with anti-tuberculosis agents. Although in most immunocompetent cases the primary infection is once controlled by immune response, the bacilli continue to exist in a dormant state in host lung and become reactivated and cause active tuberculosis when the host immune defense is hampered (1, 2). Thus, development of this disease is closely associated with the status of host immunity. The host defense against this infectious pathogen is mediated largely by cellular immune responses, and Th1 cell-mediated mechanism plays a critical role (3). In fact, IFN-γ and IL-12 are both recognized as essential cytokines for eradicating this infection (4–8). In contrast, the significance of Th2 cytokines such as IL-4 and IL-10, which are known to antagonize Th1-mediated responses (9), has not yet been substantiated because depletion of these genes did not affect the host protection against *M. tuberculosis* (10–12).

IL-18, originally discovered as an IFN-γ-inducing factor (13), triggers the production of IFN-γ by NK and Th1 cells and also promotes the cytolytic activity of NK cells (13–15). IL-18 by itself does not initiate Th1 cell development, but augments this response caused by IL-12 (16). Recently, Hoshino et al. (17) reported that IL-18 can potentially stimulate the production of Th2 cytokine by NK and T cells in vitro and its administration in vivo resulted in higher serum IL-4 and IgE levels (18). Similar findings were reported by other investigators (19, 20). Thus, IL-18 is currently recognized as a cytokine that can potentially promote both Th1 and Th2 responses (21), although the precise mechanism for determining Th phenotypes by this cytokine remains to be elucidated.

Many investigations have addressed the role of IL-18 in host defense against a variety of infectious pathogens, including *Yersinia enterocolitica* (22), *Salmonella typhimurium* (23, 24), *Leishmania major* (25–27), *Toxoplasma gondii* (28), *Plasmodium berghei* (14), and herpes simplex virus type I (29). In most studies, IL-18 was demonstrated to protect animals against infection by promoting Th1 type response. We have reported similar results indicating that endogenous IL-18 contributed to Th1 response and host resistance to *Cryptococcus neoformans* infection (30, 31), and administration of this cytokine protected mice against fatal infection (32). Sugawara et al. (33) provided data in support of the above observations by showing reduced IFN-γ synthesis and host resistance to mycobacterial infection in IL-18-deficient mice. However, the newly identified activity of IL-18 on Th2 responses has made its role in the host defense to intracellular pathogens ambiguous.

To critically evaluate the role of IL-18 in immune response and host defense against *M. tuberculosis* infection, in the present study we compared the role of IL-18 with that of IL-12 in mice lacking

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the synthesis of either IL-18 or IL-12p40 or both. Furthermore, we examined the effect of overexpression of IL-18 on such responses using mice bearing transgene for mature form of this cytokine. Our results showed a significant contribution of IL-18 to Th1 response and host resistance to this infection, although these effects were less pronounced than those of IL-12p40.

## Materials and Methods

### *Mice*

Breeding pairs of IL-12p40 gene-disrupted (knockout (KO)[3]) mice on a C57BL/6 background were obtained from The Jackson Laboratory (Bar Harbor, ME). IL-18KO mice were established as described previously (34) and back-crossed eight times to C57BL/6 mice. Mice with a deletion of the genes coding both IL-12p40 and IL-18 (double KO; DKO) were generated by mating between IL-12p40KO and IL-18KO mice. IL-18-transgenic (IL-18 Tg) mice in which B and mature T cells could express mature mouse IL-18 cDNA fused with signal peptide of the mouse Ig $\kappa$-chain under the control of mouse Ig promoter were generated, as described by Hoshino et al. (35). These mice were bred in a pathogen-free environment in the Laboratory Animal Center for Biomedical Science, University of the Ryukyus (Okinawa, Japan). C57BL/6 mice were purchased from Charles River Japan (Osaka, Japan) and used as a control wild-type (WT) animal for each KO mice. We used hemizygous IL-18 Tg mice and littermate mice without expression of the transgene as a control. All mice were used at 8–20 wk of age. All experimental protocols described in the present study were approved by the Ethics Review Committee for Animal Experimentation of our university (University of the Ryukyus, Okinawa, Japan).

### *Infection of microorganisms*

*M. tuberculosis* H37Rv strain (25618; American Type Culture Collection, Manassas, VA) from the same frozen stock was grown in Middlebrook 7H9 medium (Difco, Detroit, MI) containing 0.05% Tween 80 to mid-log phase. Mice were infected with an i.v. inoculum of *M. tuberculosis* ($1–3 \times 10^5$ CFU) suspended in 200 $\mu$l normal saline after washing three times with normal saline containing 0.05% Tween 80.

### *Enumeration of viable* M. tuberculosis

Mice were sacrificed 2 and 5 wk after infection, and spleen, lung, and liver were dissected out carefully, then separately homogenized in 10 ml of distilled water by teasing with a stainless mesh at room temperature. The homogenates, appropriately diluted with distilled water, were inoculated at 100 $\mu$l on nutrient Middlebrook 7H11 agar (Difco) plates, cultured for 2–3 wk, followed by counting the number of colonies.

### *Preparation of organ homogenates*

Mice were sacrificed before and 7, 14, and 35 days after infection; and spleen, lung, and liver were separately homogenized in 2 ml of PBS for spleen and lung or 5 ml for liver by teasing with a stainless mesh. The homogenates were centrifuged, filtered through a 0.22-$\mu$m filter (Millipore, Bedford, MA), and kept at −70°C before use.

### *Culture medium and reagents*

RPMI 1640 medium was obtained from Life Technologies (Grand Island, NY), FCS from Cansera (Rexdale, Ontario, Canada), and Con A from Sigma-Aldrich (St. Louis, MO). Purified protein derivatives (PPD) were purchased from Nihon BCG (Tokyo, Japan). Murine rIL-12 was kindly provided by Hoffmann-La Roche (Nutley, NJ).

### *In vitro stimulation of spleen cells*

Spleen cells were prepared from mice on day 14 after infection with *M. tuberculosis* and cultured at $2 \times 10^6$/ml with various doses of PPD for 48 h. The culture supernatants were collected and measured for the concentration of IFN-$\gamma$, IL-4, and IL-13 by ELISA.

### *Preparation of peritoneal exudate cells and in vitro stimulation with M. tuberculosis*

Mice were injected i.p. with 1.5 ml of 3% thioglycolate (Eiken Chemical, Tokyo, Japan), and 4 days later peritoneal exudate cells (PEC) were harvested by two cycles of injection of 5 ml of cold RPMI 1640 supplemented with 10% FCS. The obtained cells were cultured at $2 \times 10^6$/ml in the presence or absence of various doses of live *M. tuberculosis* for 48 h. The culture supernatants were collected and measured for the concentration of IL-12p40 by ELISA.

### *Measurement of cytokines*

The concentrations of IFN-$\gamma$, IL-4, IL-12p40, IL-12p70, and IL-13 in sera, organ homogenates, and culture supernatants were measured by specific ELISA kits (purchased from Endogen, Cambridge, MA) for IFN-$\gamma$ and IL-4, from BioSource International (Camarillo, CA) for IL-12p40 and p70, and from R&D Systems (Minneapolis, MN) for IL-13. The detection limits of assays for IFN-$\gamma$, IL-4, IL-12p40, IL-12p70, and IL-13 were 15, 5, 2, 4, and 10 pg/ml, respectively.

### *Statistical analysis*

Analysis of data was conducted using Statview II software (Abacus Concept, Berkeley, CA) on a Macintosh computer. Data are expressed as mean ± SD. Statistical analysis between groups was performed using the ANOVA test with a post hoc analysis (Fisher PLSD test). Survival data were analyzed using the generalized Wilcoxon test. A value of $p < 0.05$ was considered significant.

## Results

### *Contribution of IL-18 to host defense against* M. tuberculosis *infection*

To elucidate the role of IL-18 in host resistance to *M. tuberculosis* infection, we compared the susceptibility to this pathogen among IL-12p40KO, IL-18KO, DKO, and WT mice by examining the survival of infected mice and the bacterial loads of this pathogen in the spleen, lung, and liver. As shown in Fig. 1, neither WT nor IL-18KO mice succumbed to this infection during the observation periods, while all IL-12p40KO mice died within 9 wk. Interestingly, the survival time of DKO mice was significantly shorter than that of IL-12p40KO mice (mean survival time: 60.4 ± 3.6 vs 52.3 ± 6.9 days in IL-12p40KO and DKO mice, respectively; $p < 0.05$, by Fisher PLSD test), suggesting the possible contribution of IL-18 to host protection because the latter mice produced IL-18 in contrast to the former mice lacking IL-18 as well as IL-12p40.

Compatible with the above conclusion, eradication of microorganisms from the spleen was significantly delayed in IL-18KO mice, compared with that in WT mice, and in addition, the number of live bacteria in the same organ was significantly higher in DKO mice than in IL-12p40KO mice (Fig. 2*A*). Similar results were obtained in other organs, although statistical significance was detected only in the lung between WT and IL-18KO mice (Fig. 2, *B*

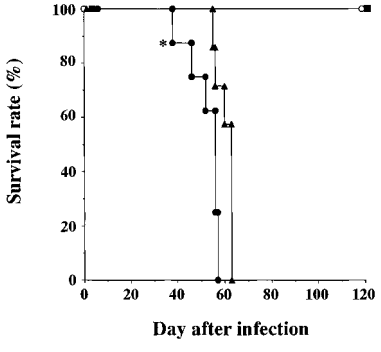


**FIGURE 1.** Comparative analyses of the survival time of WT, IL-18KO, IL-12p40KO, and DKO mice after infection with *M. tuberculosis* WT (○; $n = 8$), IL-18KO (■; $n = 8$), IL-12p40KO (▲; $n = 7$), and DKO mice (●; $n = 8$) were infected with *M. tuberculosis* ($2 \times 10^5$ CFU), and the number of live mice was noted daily. The experiments were repeated twice with similar results. *, Values of $p < 0.05$, compared with IL-12p40KO mice by the generalized Wilcoxon test.

[3] Abbreviations used in this paper: KO, knockout; DKO, double KO; IL-18 Tg, IL-18-transgenic; WT, wild type; PEC, peritoneal exudate cell; PPD, purified protein derivative.

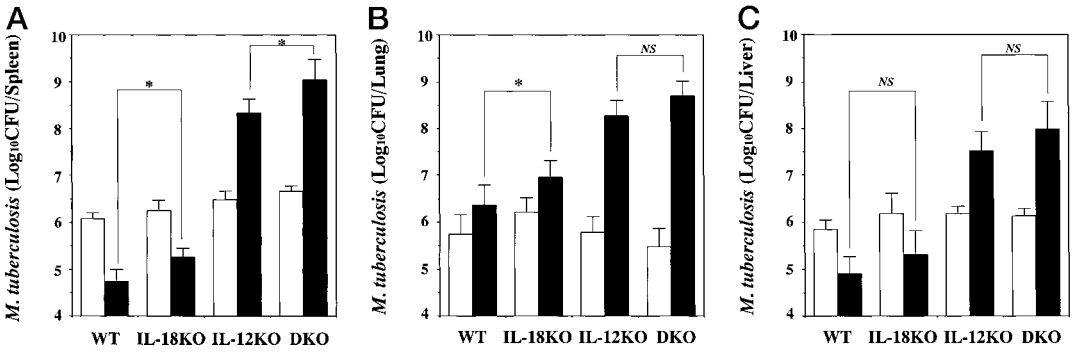


**FIGURE 2.** *M. tuberculosis* loads in the spleen, lung, and liver of WT, IL-18KO, IL-12p40KO, and DKO mice. WT, IL-18KO, IL-12p40KO, or DKO mice were infected with *M. tuberculosis* ($2.5 \times 10^5$ CFU), and the number of viable colonies in spleen (*A*), lung (*B*), and liver (*C*) was counted on days 14 (□) and 35 (■) postinfection. Each column represents the mean ± SD of six mice. The experiments were repeated three times with similar results. ∗, Values of $p < 0.05$.

and *C*). These results indicated some involvement of IL-18 in host resistance to *M. tuberculosis* infection despite the likely smaller contribution than IL-12p40.

### *Reduced Th1 response to* M. tuberculosis *in IL-18KO mice*

IL-18 was originally identified as an IFN-γ-inducing factor and known to profoundly potentiate the production of IFN-γ by various cells including NK, NKT, and Th cells (13–15). Therefore, we next examined IFN-γ production in the serum, spleen, lung, and liver of WT and IL-18KO mice before and 7, 14, and 35 days after infection with *M. tuberculosis*. As shown in Fig. 3*A–D*, IFN-γ was not detected or at small levels before infection in the serum and all organs of these mice. The IFN-γ levels increased on day 7, reached a peak level on day 14, and then decreased on day 35 after infection in WT mice. Similar kinetics were observed in IL-18KO mice, but the levels in serum, lung, and liver were significantly lower than those in WT mice at every time point, except for on day 7 in lung. In spleen, IFN-γ synthesis tended to be smaller in IL-18KO mice than in WT mice on days 7 and 14, although the differences were not statistically significant. These results indicated the significant contribution of IL-18 to the synthesis of IFN-γ induced by mycobacterial infection. In sharp contrast, serum levels of Th2 cytokines including IL-4 and IL-13 were not much different among these mouse strains at every time point after infection with *M. tuberculosis* (data not shown).

In additional experiments, we examined the effects of deficiency of IFN-γ-inducing cytokines on the development of Th1 cells during *M. tuberculosis* infection by measuring the synthesis of IFN-γ by spleen cells from infected mice upon restimulation with PPD. As shown in Fig. 4, IFN-γ production by spleen cells was abrogated in both IL-12p40KO and DKO mice, while a significant reduction, but not abrogation, was observed in IL-18KO mice. These results suggested that IL-18 played an important role, although not essential, in the differentiation of mycobacterial Ag-specific Th1 cells. In contrast, there was no clear distinction in Th2 cell development, as indicated by spleen cell production of IL-13 among WT, IL-12p40KO, IL-18KO, and DKO mice, although IL-4 production was not detected at any doses of PPD (data not shown).

### *Reduced IL-12 synthesis and effect of its replacement on the production of IFN-γ in IL-18KO mice*

To examine the effect of IL-18 deficiency on IL-12 synthesis, we compared the serum levels of IL-12p40 and p70 between WT and

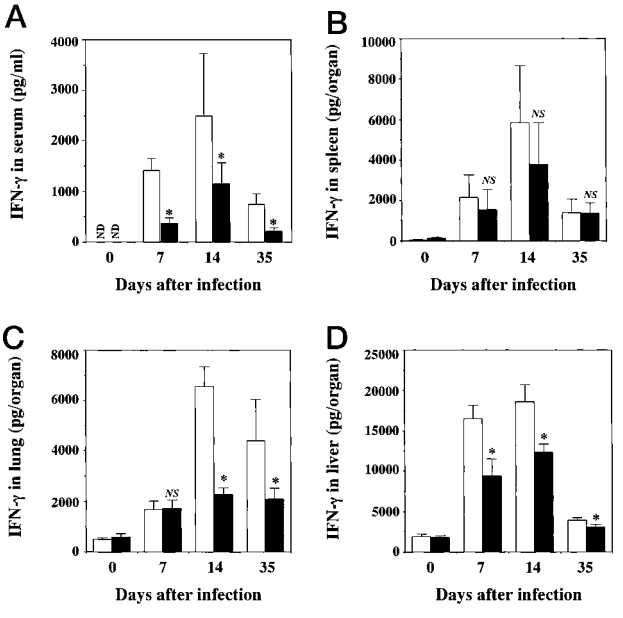


**FIGURE 3.** Reduced production of IFN-γ in *M. tuberculosis*-infected IL-18KO mice WT and IL-18KO mice were infected with *M. tuberculosis* ($1.7 \times 10^5$ CFU). IFN-γ in serum (*A*) and homogenates of spleen (*B*), lung (*C*), and liver (*D*) were measured before and 7, 14, and 35 days after infection. Each column represents the mean ± SD of six mice. The experiments were repeated twice with similar results. □, WT; ■, IL-18KO. ND, Not detected. ∗, Values of $p < 0.05$, compared with WT mice.

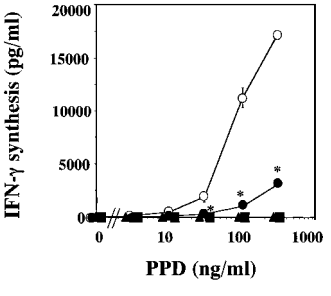


**FIGURE 4.** Reduced spleen cell production of IFN-γ upon restimulation with PPD in *M. tuberculosis*-infected IL-18KO mice WT, IL-18KO, IL-12p40KO and DKO mice were infected with *M. tuberculosis* ($2 \times 10^5$ CFU). Spleen cells, pooled from four mice on day 14 postinfection, were stimulated with indicated doses of PPD for 48 h, and concentration of IFN-γ in the culture supernatants was measured. Each symbol represents the mean ± SD of triplicate cultures. The experiments were repeated twice with similar results. ○, WT; ●, IL-18KO; ▲, IL-12p40KO; ■, DKO. ∗, Values of $p < 0.05$, compared with WT mice.

IL-18KO mice after infection with *M. tuberculosis*. As shown in Fig. 5*A*, IL-12p40 levels were significantly lower in IL-18KO mice than in WT mice on days 7, 14, and 35 postinfection, although IL-12p70 was under detection limit at every time point (data not shown). In additional experiments, we examined the production of IL-12p40 by PEC upon stimulation with live *M. tuberculosis* in these mice. Similarly, the in vitro synthesis of this cytokine was significantly reduced in IL-18KO mice, when compared with that in WT mice (Fig. 5*B*). These results suggested that the attenuated IFN-γ synthesis in IL-18KO mice may be secondary to insufficient production of IL-12 rather than lack of IL-18. To confirm this possibility, we examined the effect of replacement therapy using rIL-12 on the serum IFN-γ concentrations in infected IL-18KO mice. As shown in Fig. 6, the serum levels of IFN-γ was strongly reduced in IL-18KO mice, compared with WT mice on day 14 after mycobacterial infection. The attenuated IFN-γ level increased by treatment with rIL-12 in IL-18KO mice, but such increase was quite partial and did not reach the comparable level to that in WT mice even when an excess dose of rIL-12 was used. In contrast, the same treatment completely restored the lacked synthesis of IFN-γ in IL-12p40KO mice (data not shown). These results supported the hypothesis that IL-18 as well as IL-12 contributed to the Th1 response induced by *M. tuberculosis* infection.

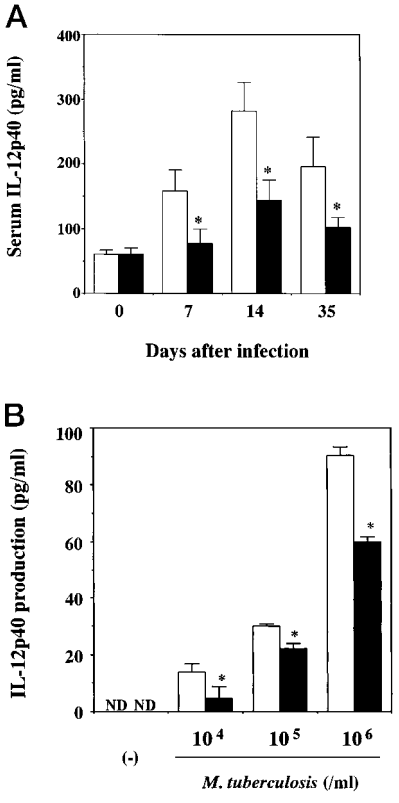


**FIGURE 5.** Reduced production of IL-12 in *M. tuberculosis*-infected IL-18KO mice (*A*) WT or IL-18KO mice were infected with *M. tuberculosis* ($2 \times 10^5$ CFU), and serum levels of IL-12p40 were measured before and 7, 14, and 35 days after infection. Each column represents the mean ± SD of six mice. *B*, PEC from WT or IL-18KO mice were cultured at $2 \times 10^6$/ml with indicated numbers of live *M. tuberculosis* for 48 h, and concentrations of IL-12p40 in the culture supernatants were measured. Each symbol represents the mean ± SD of triplicate cultures. The experiments were repeated three (*A*) or two times (*B*) with similar results. □, WT; ■, IL-18KO. ND, Not detected. ∗, Values of $p < 0.05$, compared with WT mice.

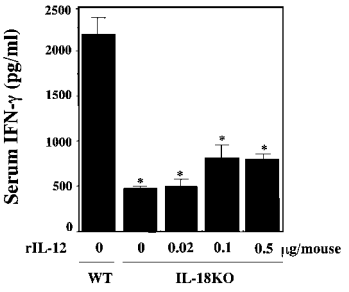


**FIGURE 6.** Failure of rIL-12 to increase IFN-γ production in *M. tuberculosis*-infected IL-18KO mice WT and IL-18KO mice were infected with *M. tuberculosis* ($2 \times 10^5$ CFU), and the latter mice received daily i.p. injections of rIL-12 at indicated doses for 7 days after infection. Serum levels of IFN-γ were measured on day 14. Each column represents the mean ± SD of six mice. The experiments were repeated twice with similar results. ∗, Values of $p < 0.05$, compared with WT mice.

### *Host defense and Th1 response to* M. tuberculosis *in IL-18 Tg mice*

To further confirm the role of IL-18 in the protective response against mycobacterial infection, we examined the effect of IL-18 overproduction on the host defense and Th1 response to this pathogen using mice expressing the transgene for this cytokine. As shown in Table I, the number of live bacteria was significantly reduced in the spleen and in the spleen and lung of IL-18 Tg mice, compared with control mice, on days 14 and 35 after infection, respectively.

To define the mechanism of the reduced susceptibility of IL-18 Tg mice to infection, we examined the production of IFN-γ, IL-4, and IL-13 in the two strains of mice. Before infection, all of these cytokines were detected in the serum of IL-18 Tg, as shown in Fig. 7, but not of control mice. Serum IFN-γ was increased at 3-, 7-, and 20-fold higher levels in IL-18 Tg mice than in control mice on days 7, 14, and 35 after infection with *M. tuberculosis*, respectively (Fig. 7*A*). In contrast, IL-4 and IL-13 in serum were not much different in these mice at the same time intervals, except for on day 14 postinfection when IL-13 production was significantly higher in IL-18 Tg mice (Fig. 7, *B* and *C*).

Finally, to approach the effect of overproduction of IL-18 on the differentiation of *M. tuberculosis*-specific Th1 and Th2 cells, we compared the production of IFN-γ, IL-4, and IL-13 by spleen cells upon restimulation with various amounts of PPD between IL-18 Tg and control littermate mice on day 14 after infection. As shown in Fig. 8*A*, IFN-γ production by spleen cells restimulated with PPD was significantly higher at each dose except for 300 ng/ml in IL-18 Tg mice than in control mice. Interestingly, IL-13 production was significantly increased in IL-18 Tg mice, when compared

Table I. *Effect of IL-18 overproduction on M. tuberculosis infection*[a]

| Organs | Day 14 | | Day 35 | |
|---|---|---|---|---|
| | CNT[b] | IL-18 Tg[c] | CNT | IL-18 Tg |
| Spleen | 5.8 ± 0.2[d] | 5.0 ± 0.2[e] | 4.4 ± 0.2 | 3.8 ± 0.4[e] |
| Lung | 4.0 ± 0.4 | 3.7 ± 0.7[f] | 5.1 ± 0.5 | 4.3 ± 0.4[e] |
| Liver | 5.2 ± 0.3 | 5.3 ± 0.3[f] | 4.3 ± 0.4 | 3.9 ± 0.3[f] |

[a] Mice were infected with *M. tuberculosis* ($3 \times 10^5$ CFU) and the bacterial loads in spleen, lung, and liver were examined on days 14 and 35.
[b] Control littermate mice.
[c] IL-18 Tg mice.
[d] Mean $\log_{10}$ CFU/organ ± SD of six mice.
[e] Values of $p < 0.05$, compared to control mice.
[f] NS, compared with control mice.

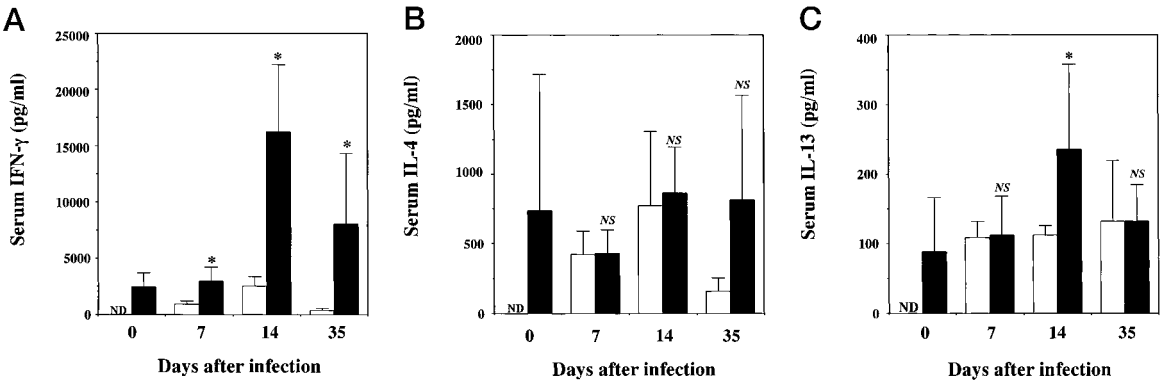


**FIGURE 7.** Effect of IL-18 overproduction on serum levels of Th1 and Th2 cytokines after infection with *M. tuberculosis* IL-18 Tg (■) and control littermate mice (□) were infected with *M. tuberculosis* ($3 \times 10^5$ CFU), and serum levels of IFN-γ (*A*), IL-4 (*B*), and IL-13 (*C*) were measured before and 7, 14, and 35 days after infection. Each column represents the mean ± SD of six mice. The experiments were repeated twice with similar results. ND, Not detected. *, Values of $p < 0.05$, compared with control mice.

with that in control mice (Fig. 8*B*), although IL-4 was not detected at any doses of PPD (data not shown).

## Discussion

IL-18 was originally identified as a Th1-related cytokine, which induced IFN-γ production by Th and NK cells (13–15) and potentiated IL-12-induced development of Th1 cells (16). However, recent studies have reported the involvement of IL-18 in the induction of Th2 response in the absence of IL-12 production (17–21). Such ambiguous effects have made the role of this cytokine in the host defense against intracellular infectious pathogens less clear. In the present study, we carefully elucidated the role of IL-18 in Th1 response and host resistance against *M. tuberculosis* infection by using mice lacking or overproducing this cytokine, and furthermore by comparing the cytokine responses and clinical course of infection among mice with disrupted genes for IL-12p40 and/or IL-18. The major findings of our study were: 1) IL-18-deficiency rendered mice slightly prone to mycobacterial infection through reduction of IFN-γ production, but not much affecting the Th2 response; 2) mice lacking both IL-12p40 and IL-18 were more susceptible to this infection than IL-12p40-deficient mice; 3) IL-18 Tg mice were more resistant to infection through enhancing the production of IFN-γ.

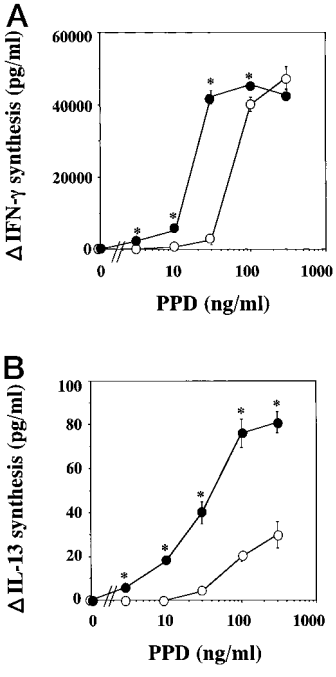


**FIGURE 8.** Effects of IL-18 overproduction on synthesis of Th1 and Th2 cytokines by restimulated spleen cells IL-18 Tg (●) or control littermate mice (○) were infected with *M. tuberculosis* ($1 \times 10^5$ CFU). Spleen cells, pooled from four mice on day 14 postinfection, were stimulated with indicated doses of PPD for 48 h, and concentration of IFN-γ (*A*) and IL-13 (*B*) in the culture supernatants was measured. The results were expressed as the values subtracted by that of unstimulated spleen cells (55 ± 1 and 3999 ± 463 pg/ml for IFN-γ and not detected and 17 ± 1 pg/ml for IL-13 in control and IL-18 Tg mice, respectively). Each symbol represents the mean ± SD of triplicate cultures. The experiments were repeated twice with similar results. *, Values of $p < 0.05$, compared with control mice.

Sugawara et al. (33) demonstrated previously no significant difference in the survival of IL-18KO and WT mice infected with i.v. administration of *M. tuberculosis*, but the former mice had a limited ability in eradicating the airborne infection than the latter mice, as indicated by the increased number of live bacilli in lungs. Our present study extended their observations by making comparative analyses with mice lacking either IL-12p40 or IL-18 or both. Under IL-18-deficient condition, the survival of mice infected with i.v.-administered *M. tuberculosis* was similar to control mice. However, there was a significant difference in the survival time between IL-12p40KO and DKO mice: the latter mice deficient in IL-12p40 and IL-18 synthesis died earlier than the former mice lacking IL-12p40, but not IL-18. Compatible with these results, eradication of the pathogen from the spleen and lung was slightly delayed in IL-18KO mice, when compared with that in WT mice, and the number of live colonies in the spleen was higher in DKO mice than in IL-12p40KO mice. Although such a difference was quite small, we would like to think there is some biological significance, because similar results were reproducibly obtained in three independent experiments. Similar findings were recently reported by our laboratory (31). Elimination of *C. neoformans* from the infected site was delayed in IL-18KO mice when compared with that in WT mice, and DKO mice were more sensitive to this infection than IL-12p40KO mice. Other investigators also indicated the critical roles of IL-18 in the host resistance to various infectious pathogens (22–29), but there are no studies that have compared its role with other IFN-γ-inducing cytokines apart from ours. Recently, IL-23, a novel cytokine which shares IL-12p40 and some biological activities with IL-12, has been identified (36). This cytokine induces IFN-γ production by T cells, similar

to IL-12 and IL-18. Therefore, it should be noted that IL-12p40KO mice, which were used in the present study to examine the effect of IL-12-deficient condition, lacked IL-23 as well as IL-12, as recently indicated by Cooper et al. (37). Thus, our present data suggested the significant contribution of IL-18, although much less pronounced than IL-12p40, to the host defense against mycobacterial infection, similar to cryptococcal infection.

IL-12 is essential for the differentiation of OVA-specific Th1 cells from naive Th cells, while IL-18 by itself cannot initiate, but potentiate this response (16). A similar role of IL-18 was reported during the development of immune responses against infection with *C. neoformans* (31). Spleen cells from IL-18KO mice infected with this fungal microorganism produced smaller amounts of IFN-$\gamma$ upon restimulation with Ags than those from WT mice, while such production was not detected in IL-12p40KO and DKO mice. These previous observations are compatible with the present data showing that Th1 cell development was attenuated in IL-18KO mice and abrogated in both IL-12p40 and DKO mice during *M. tuberculosis* infection. In addition, our data depicting that serum IFN-$\gamma$ levels were reduced in IL-18KO mice and not detected in other two mice (data not shown) were in concordance with the above scenario. Taken together, IL-18 was likely to play an unignorable role in the development of mycobacteria-specific Th1 cells through potentiating, rather than initiating, such response. In fact, IL-18 Tg mice showed higher levels of IFN-$\gamma$ in sera and showed a higher production of IFN-$\gamma$ by spleen cells upon restimulation with mycobacterial Ags than control littermate mice.

Serum concentrations of IL-12 were significantly reduced in IL-18KO mice, compared with WT mice, at every time point after infection with *M. tuberculosis*. PEC from IL-18KO mice produced less IL-12 upon in vitro infection with this bacilli than those from WT mice. Similar results were obtained in IL-12 synthesis by the spleen cells from infected mice (data not shown). Such attenuated production of IL-12 under IL-18-deficient condition was also observed by other investigators (30, 38, 39), although the precise mechanism remains to be elucidated. These findings implied an alternative mechanism; the lower level of IFN-$\gamma$ synthesis in IL-18KO mice was due to the reduced production of IL-12, but not due to deficient IL-18 secretion. In fact, IL-12 provides a much more potent influence on the induction of Th1 response than IL-18 (16, 31, 34). However, this was not likely the case in our study, because excess amounts of rIL-12 did not restore the reduced production of IFN-$\gamma$ in the sera of IL-18KO mice with mycobacterial infection. The same treatment induced serum IFN-$\gamma$ production in IL-12p40KO mice to the comparable level to that in WT mice (data not shown). Thus, IL-18 effects on the Th1 response in mycobacterial infection did not appear to be merely secondary events of IL-12.

In other alternative mechanisms, expression of IL-12 receptor may be lowered in T and NK cells from IL-18KO mice, compared with WT mice. To address this possibility, we compared the responsiveness of cells to IL-12 by measuring the proliferative response and IFN-$\gamma$ production of spleen cells cultured with or without rIL-12 in the presence of suboptimal Con A. There was no significant difference in both responses between IL-18KO and WT mice (data not shown). These results indicated that the above possibility was not likely to explain the reduced IFN-$\gamma$ synthesis observed in IL-18KO mice, although we have not directly examined IL-12 receptor expression on these cells.

A new aspect of IL-18 has recently been reported by several investigators. In contrast to its originally recognized action, they found IL-18 was potent in inducing Th2 cytokine production in vitro by NK, T cells, and basophils under particular conditions (17–21). In vivo administration of this cytokine resulted in the progression of Th2 responses including eosinophilic infiltration and elevation of IgE and IgG1 in serum (18–21). Consistent with these findings, IL-18 Tg mice established by Hoshino et al. (35) spontaneously produced Th2 as well as Th1 cytokines and developed Th2-type responses with their growth, which indicated the dual role of this cytokine in determining Th phenotypes. Interestingly, in our study, IL-18 Tg mice developed predominantly Th1 response, as shown by increased IFN-$\gamma$ production, and a high resistance to *M. tuberculosis* infection, suggesting that the committed Th2 response might be shifted toward Th1-dominant condition during this infection, although IL-13 production was still higher in these mice. Compatible with this observation, both Th1 response and host resistance to this microorganism were reduced in IL-18KO mice when compared with WT mice, while Th2 response did not receive significant effect in the former mice. Based on these data, IL-18 seems to contribute primarily to the development of Th1 response rather than Th2 by yet unknown regulatory mechanism during infection with *M. tuberculosis*.

In conclusion, the present study addressed more critically the role of IL-18 in host protective response against *M. tuberculosis*. On the basis of data from comparative analyses with gene-disrupted mice for IL-12p40 and/or IL-18 and experiments using IL-18 Tg mice, significant contribution of IL-18 to Th1, rather than Th2 response and host resistance to this infection were established, although these effects were less pronounced than those of IL-12. Importantly, previous studies (8) as well as ours depicting the role of IL-12 in host defense against mycobacterial infection used mice lacking IL-12p40; and therefore, such studies cannot discriminate the role of IL-12 from that of IL-23. Interestingly, a recent study by Cooper et al. (37) have speculated the putative role of this novel cytokine in host protective responses to mycobacterial infection under a condition lacking the synthesis of bioactive IL-12. Further investigations simultaneously using mice deficient of either IL-12p35 or IL-23p19 are necessary to understand the critical role of each IFN-$\gamma$-inducing cytokine in the host defense.

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