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Genetically Resistant Mice Lacking IL-18 Gene Develop Th1 Response and Control Cutaneous Leishmania major Infection

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IL-18 has been shown to play a critical role in the development of a Th1 response and immunity against intracellular pathogens. To determine the role of IL-18 in the development of protective immunity against Leishmania major, we have analyzed the course of cutaneous L. major in IL-18-deficient C57BL/6 mice (IL-18−/−) compared with similarly infected wild-type mice (IL-18+/+). After L. major infection, IL-18−/− mice may develop larger lesions during early phase of infection but eventually will resolve them as efficiently as IL-18+/+ mice. By 2 wk after infection, although Ag-stimulated lymph node cells from L. major-infected IL-18+/+ and IL-18−/− mice produced similar levels of IFN-γ, those from IL-18−/− mice produced significantly more IL-12 and IL-4. By 10 wk after infection, both IL-18+/+ and IL-18−/− mice had resolved L. major infection. At this time, lymph node cells from both IL-18+/+ and IL-18−/− mice produced IL-12 and IFN-γ but no IL-4. Furthermore, administration of anti-IFN-γ Abs to IL-18−/− mice rendered them susceptible to L. major. These results indicate that despite the role IL-18 may play in early control of cutaneous L. major lesion growth, this cytokine is not critical for development of protective Th1 response and resolution of L. major infection. The Journal of Immunology, 2000, 164: 5890–5893.

Leishmania are obligate intracellular parasites that cause a wide range of diseases such as cutaneous, mucocutaneous, and visceral leishmaniasis (1). The murine model of cutaneous Leishmania major infection has been well characterized and frequently has been used as a functional model of Th1 and Th2 cell responses (2). Control of cutaneous L. major infection in resistant mice such as CHL and C57BL/6 is associated with the development of IL-12-induced Th1-type response and the production of IFN-γ (2–4). In contrast, susceptible BALB/c mice develop large nonhealing lesions after L. major infection and mount a Th2-type response that is associated with the production of the cytokines IL-4 and IL-10 (2, 5).

IL-18 is a recently discovered cytokine that is produced by activated macrophages (6). This cytokine has been shown to play a critical role in the development of protective immunity against intracellular pathogens including Mycobacterium tuberculosis, Cryptococcus neoformans, Yersinia enterocolitica, and acute HSV type 1 (7–10). The protective role of IL-18 in these infections has been attributed to its ability to activate NK cells, enhance proliferation of activated T cells, and induce IFN-γ production. Moreover, one study using IL-12/IL-18−/− (double mutant) mice has demonstrated that both IL-12 and IL-18 act in synergy to activate NK cells and induce Th1 development in vivo (11).

Several studies have demonstrated that IL-12 is indispensable for the development of protective immunity against L. major (12, 13). A recent study also found that susceptible BALB/c mice treated with recombinant IL-18 required only small quantities of IL-12 to control cutaneous L. major infection (14). Although these results demonstrate that exogenously administered IL-18 may act synergistically with IL-12 to induce protection against L. major infection in susceptible BALB/c mice, it is not clear whether endogenous IL-18 plays a similar role in the development of protective immunity against L. major in resistant mice. Therefore, we examined the development of Th1 response and cutaneous growth of L. major in resistant C57BL/6 mice lacking the IL-18 gene. Our results suggest that IL-18 may be involved in controlling early lesion growth but that it is not required for the development of a Th1 response and the resolution of L. major infection.

Materials and Methods

Animals

IL-18 gene-deficient C57BL/6 mice were generated as described previously (11) and were kindly provided by Dr. S. Akira (Osaka University, Osaka, Japan). The mice were bred and maintained in the facility at the Harvard School of Public Health (Boston, MA) according to the guidelines for animal research. Wild-type C57BL/6 and IL-12−/− C57BL/6 mice of the same sex and age were purchased from The Jackson Laboratory (Bar Harbor, ME) and were used as controls in all experiments.

Parasites and infection protocols

L. major LV39 was maintained by serial passage of amastigotes inoculated s.c. into the shaven rumps of BALB/c mice as described previously (15). Groups of C57BL/6 IL-18 gene-deficient and wild-type C57BL/6 mice were infected in the right hind footpad with 2 × 10⁶ stationary-phase promastigotes of L. major (LV39). Lesion development was measured using a dial-gauge micrometer (Mitutoyo, Kanagawa, Japan) at weekly intervals up to 10 wk after infection. The increase in the thickness of the right hind footpad was compared with the uninfected left hind footpad.

T cell proliferation assay and cytokine analysis

The draining popliteal lymph nodes were removed from L. major-infected mice at 2 and 10 wk after infection. T cell proliferation assays were performed as previously described (15). To the wells of a 96-well flat-bottom
significant differences between groups (p described previously (15). Cultures were analyzed for production of IFN-

IL-18 2 2 experiment we compared lesion development in IL-12 to the thickness of the uninfected left footpad. 

suring the increase in lesion size of the infected footpad and comparing it 

to the thickness of the uninfected left footpad. C, In the third independent 

experiment we compared lesion development in IL-12 /-/- mice to that of 

IL-18 /-/- and IL-18 +/- mice. In all three experiments, IL-18 /-/- mice de-

dveloped larger lesions in early course of infection than IL-18 +/- mice did; 

however, the differences in lesion sizes were significant in experiment 2 

only (B). Data are presented as mean lesion size ± SE. *, Statistically 
significant differences between groups (p < 0.05).

FIGURE 1. Course of L. major infection in IL-18 +/- and IL-18 /-/- mice. A and B. In two independent experiments IL-18 +/- and IL-18 /-/- mice were inoculated with 2 x 10^6 L. major stationary-phase promastigotes into the right hind footpad, and lesion growth was monitored by measuring the increase in lesion size of the infected footpad and comparing it to the thickness of the uninfected left footpad. C. In the third independent experiment we compared lesion development in IL-12 /-/- mice to that of IL-18 /-/- and IL-18 +/- mice. In all three experiments, IL-18 /-/- mice developed larger lesions in early course of infection than IL-18 +/- mice did; however, the differences in lesion sizes were significant in experiment 2 only (B). Data are presented as mean lesion size ± SE. *, Statistically significant differences between groups (p < 0.05).

Histopathology

Infected footpads from IL-18 /-/- and IL-18 +/- mice were removed and 

fixed in decalifying solution F (Stephens Lab, Riverdale, NJ) for 7 days. The tissues were processed and embedded in paraffin, and 4- to 8-µm sections were cut. The sections were hydrated and stained by routine hema
toxylin and eosin staining.

Anti-IFN-γ neutralizing Ab treatment 

Rat anti-mouse IFN-γ (clone, XMG 1.2) neutralizing mAb was kindly 

provided by Dr. Mary Russell. IL-18 /-/- mice were treated by i.p. admin-

istration of 1 mg anti-IFN-γ neutralizing Ab or control Ab 1 day before 

L. major infection and a weekly dose of 1 mg/mouse thereafter for 7 wk.

Statistical significance

Student’s unpaired t test was used to determine statistical significance of 

values obtained. Differences in Ab endpoint titers were determined using the Mann-Whitney U prime test.

Results and Discussion

The results presented in this study suggest that IL-18 may be in-

volved in controlling early L. major lesion growth in resistant 

C57BL/6 mice but that it is not essential for the development of 

acquired protective immunity and resolution of L. major infection. In addition, these findings also demonstrate that the development of larger lesions in IL-18 /-/- mice in early course of infection is associated with a significant increase in IL-4 production rather than a decrease in IFN-γ.

Previous studies have demonstrated that IL-12 plays a critical role in mediating protective immunity against L. major (14, 17, 18). The protective role of IL-12 in murine L. major infection has been attributed to its ability to activate NK cells and induce IFN-γ production required to develop a Th1 cell response (19). However, we have recently demonstrated that endogenous IL-12 can directly induce protective Th1-like response in the absence of NK cells and can control L. major infection (15). IL-18 is a recently discovered cytokine that is produced by activated macrophages (6, 20). This cytokine shares immunoregulatory functions with IL-12 and plays a critical role in the host defense against several pathogens (8, 9, 19, 21–23). A recent study has demonstrated that the treatment of susceptible BALB/c mice with exogenous IL-12 together with small quantities of IL-12 significantly enhanced their resistance to cutaneous L. major infection, indicating that IL-18 may play a role in the development of protective immunity against L. major (14). In the present study, we found that IL-18 /-/- C57BL/6 mice, although they occasionally developed significantly larger lesions

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5 Abbreviation used in this paper: LmAg, freeze-thawed L. major Ag.
during the early course of infection (Fig. 1B), eventually resolved their lesions (Fig. 1, A–C) and controlled parasite replication (Fig. 2). Furthermore, infected footpads from both IL-18+/+ and IL-18−/− mice displayed preserved skin and an inflammatory infiltrate comprised of lymphocytes and macrophages with few or no parasites. In contrast, in another replicate experiment, concomitantly infected IL-12−/− C57BL/6 mice developed large nonhealing lesions by week 6 after infection (Fig. 1C). The lesions from IL-12−/− mice showed significant ulceration of skin with necrosis and inflammatory infiltrate comprised primarily of heavily parasitized macrophages, neutrophils, and eosinophils. Our findings contradict a recent study that demonstrated that IL-18−/− mice on CD1 genetic background are highly susceptible to \emph{L. major} and develop large nonhealing lesions by day 40 after infection (24). Although different results observed in our study and that by Wei et al. (24) can be attributed to the differences in genetic backgrounds of the strains used, we also monitored disease progression for a longer duration. As reported by Wei et al., \emph{L. major}-infected IL-18−/− mice did develop larger lesions than infected IL-18+/+ mice did during early course of infection but eventually resolved them (Fig. 1, A–C). Moreover, the difference in early lesion sizes between IL-18−/− and IL-18+/+ mice was statistically significant in only one of the three experiments (Fig. 1B).

Previous studies indicate that IL-12-activated NK cells are the primary source of IFN-γ early in \emph{L. major} infection that is required for the development of a Th1 response in resistant mice (19). Recent studies have demonstrated that NK cells and the Th1 subset of CD4+ T cells express IL-18R (25). Furthermore, NK cell activity is also significantly impaired in IL-18−/− mice (11). Therefore, we measured IL-12 and IFN-γ production by LmAg-stimulated lymph node cells and also determined the serum levels of LmAg-specific IgG2a Abs in IL-18+/+ and IL-18−/− mice at 2 and 10 wk after \emph{L. major} infection. At both of these time points, LmAg-stimulated lymph node cells from both IL-18+/+ and IL-18−/− mice produced comparable levels of IFN-γ (Fig. 3A), and both groups displayed significant titers of Ag-specific IgG2a Abs at these time points (Fig. 4). Furthermore, \emph{L. major}-infected IL-18−/− mice treated with anti-IFN-γ neutralizing Ab developed significantly larger lesions compared with those on similarly infected IL-18−/− mice treated with control Ab (Fig. 5). These results in-

**FIGURE 3.** Kinetics of in vitro LmAg (20 μg/ml)-induced IFN-γ (A), IL-12 (B), and IL-4 (C) production by popliteal lymph node cells from \emph{L. major}-infected IL-18+/+ and IL-18−/− mice. The data are the mean of six to seven animals at each time point for two of the three experiments. *, Statistically significant differences between each group \((p < 0.05)\).

**FIGURE 4.** Ab responses in \emph{L. major}-infected IL-18+/+ and IL-18−/− mice at 2 wk and 10 wk after infection. A, LmAg-specific IgG1; and B, Lm-Ag-specific IgG2a. Data for IgG1 and IgG2a are presented as mean reciprocal endpoint titer on log scale. Three to five mice were analyzed in each group for two of the three experiments.

**FIGURE 5.** Administration of IFN-γ neutralizing Ab to IL-18−/− mice exacerbates cutaneous \emph{L. major} infection. Data are expressed as mean lesion size ± SE. *, Statistically significant differences between each group \((p < 0.05)\).
dicate that IL-12 alone in the absence of endogenous IL-18 induces protective Th1 response and controls L. major infection. This is perhaps not surprising because we recently demonstrated that IL-12 can directly induce Th1 development in mice lacking NK cells and can control L. major infection (15). Interestingly, at 2 wk after infection, lymph node cells from IL-18−/− mice produced significantly more IL-12 than those from IL-18+/+ mice (Fig. 3B). These findings indicate that IL-18 may be involved in down-regulation of IL-12 production as suggested previously (24).

IL-4 has been shown to play a role in mediating susceptibility to cutaneous L. major infection. The disease-exacerbating role of IL-4 in cutaneous L. major infection has been attributed to its ability to induce Th2 development, inhibit Th1 development, and directly inhibit macrophage leishmanicidal activity (1). In this study, LmAg-stimulated lymph node cells from IL-18−/− mice produced significantly greater amounts of IL-4 at 2 wk after infection than those from IL-18+/+ mice (Fig. 3C). However, IL-4 was undetectable in lymph node cell culture supernatants from either group at 10 wk after infection. These results indicate that although increased IL-4 production during early phase of L. major infection may contribute to the development of large lesions in IL-18−/− mice during early course of infection, it is not sufficient to induce Th2 differentiation and to render IL-18−/− highly susceptible to L. major infection.

In conclusion, IL-18−/− mice from a resistant C57BL/6 genetic background may develop larger lesions during early course of L. major infection but eventually will resolve them efficiently. Moreover, the resolution of L. major infection by IL-18−/− mice is associated with the development of an efficient Th1 response.

References