

IL-12-induced IFN- γ is dependent on caspase-1 processing of the IL-18 precursor

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IL-12 and IL-18 are IFN- γ -inducing cytokines. In the present study, the role of endogenous IL-18 in the induction of IFN- γ by IL-12 was investigated in mice. In the presence of a specific inhibitor of caspase-1 (also known as IL-1 β -converting enzyme, or ICE) IL-12-induced IFN- γ from splenocytes was reduced by 85%. Using splenocytes from ICE-deficient mice, IL-12-induced IFN- γ was reduced by 80%. However, the role of ICE was not through processing and release of IL-1 β . Neutralizing anti-IL-18 IgG reduced IL-12-induced IFN- γ in splenocytes by 85%. Splenocytes cultured *in vitro* spontaneously released IL-18 into the extracellular compartment over time. Extracellular levels of IL-18 significantly correlated with IL-12-induced IFN- γ and were reduced in cells obtained from ICE-deficient mice. *In vivo*, IL-12 administration increased circulating levels of IL-18 in wild-type mice but not in ICE-deficient mice. Both neutralization of IL-18 and ICE deficiency significantly reduced induction of circulating IFN- γ in mice receiving IL-12. The IL-18 precursor was constitutively expressed in the livers and spleens of untreated mice. Furthermore, administration of IL-12 significantly increased liver-associated IL-18 levels. These data demonstrate that endogenous, ICE-cleaved IL-18 significantly contributes to induction of IFN- γ by IL-12.

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Introduction

Two cytokines, IL-12 and IL-18, are currently regarded as the primary inducers of IFN- γ production in an inflammatory reaction (1, 2). However, the relationship between these 2 cytokines is still not fully understood.

IL-12 is a heterodimeric cytokine produced mainly by monocytes/macrophages. In addition to inducing IFN- γ , IL-12 stimulates the production of other cytokines, activates natural killer (NK) and T cells, and promotes the development of T-helper type 1 (Th1) responses (1). Administration of IL-12 to mice results in marked splenomegaly, thymic atrophy, macrophage infiltration in tissues, and induction of IFN- γ and TNF- α synthesis (3-5). Most of the toxic effects of IL-12 are due to its ability to induce high levels of IFN- γ (3). Systemic toxicity has also been observed in cancer patients treated with multiple doses of IL-12 (6).

Like IL-12, IL-18 is a monocyte/macrophage-derived cytokine that participates in the induction of IFN- γ and other cytokines (2). IL-18 is structurally related to IL-1 β (7). Similar to the IL-1 β precursor, the IL-18 precursor (pro-IL-18) also lacks a signal peptide and requires caspase-1 (also known as IL-1 β -converting enzyme, or ICE) for cleavage and release of the mature molecule from the intracellular compartment (7-9). Only mature IL-18 is bioactive, whereas pro-IL-18 is biologically inactive (10). Therefore, mice deficient in ICE (ICE KO) have a defect in the production and release of mature, bioactive IL-18, whereas the precursor form is normally synthesized (8). The importance of the presence of both IL-12 and IL-18 for optimal

induction of IFN- γ has been demonstrated in IL-18 and ICE KO mice (11-13).

In the absence of a costimulus, IL-18 is a weak inducer of IFN- γ . However, a synergy for IFN- γ production is observed when cells are cultured with IL-18 in the presence of costimuli (14-16). Different mechanisms may account for the synergy between IL-12 and IL-18. In particular, IL-12 upregulates the expression of the IL-18 receptor, therefore rendering cells more sensitive to IL-18 (17, 18). In addition, IL-12 and IL-18 regulate the transcriptional activity of the IFN- γ promoter at different levels (19), thus providing 2 distinct signals to the IFN- γ -producing cell. IL-12 and IL-18 also regulate each other's production. (20, 21).

In the present report, we investigated the role of IL-18 in the induction of IFN- γ by IL-12. IL-12-induced IFN- γ production was evaluated both *in vitro* and *in vivo* in the presence of neutralizing anti-IL-18 antibodies. In addition, a specific ICE inhibitor and ICE KO mice were used to evaluate the role of this enzyme in the production and release of mature, bioactive IL-18 in response to IL-12. Finally, we measured levels of IL-18 both *in vitro* and *in vivo* in the presence or absence of IL-12 stimulation.

Methods

Reagents and mice. Murine recombinant IL-12 was a kind gift of Genetics Institute Inc. (Andover, Massachusetts, USA). The specific activity of IL-12 was 2.7×10^6 U/mg. Human recombinant IL-1 receptor antagonist (IL-1Ra) was a kind gift of Daniel Tracey (Upjohn Co., Kalamazoo, Michigan, USA). The reversible ICE inhibitor Ac-Tyr-Val-

Asp-2,6-dimethylbenzoyloxymethylketone was purchased from Alexis Corp. (San Diego, California, USA). RPMI and FBS were from Life Technologies Inc. (Grand Island, New York, USA). The anti-murine CSF antibody was from Endogen Inc. (Woburn, Massachusetts, USA).

The generation and genetic background of ICE KO mice have been described previously (22). Six- to 8-week old female mice were used. The wild-type (WT) mice used were of the same genetic background, sex, and age as the ICE KO mice, although they were not littermates.

In vivo experimental studies. Studies were approved by the Animal Use and Care Committee at the University of Colorado Health Sciences Center. Mice were injected intraperitoneally with either 100 or 400 ng of murine recombinant IL-12 daily for 4 days, between 0900 and 1100 hours. Control groups received 4 daily injections of vehicle (PBS [pH 7.4] containing 0.1% BSA). In some experiments, mice were injected with 200 μ L of a neutralizing rabbit anti-murine IL-18 antiserum (13) 1 hour before the first and the third injection of IL-12. A group of mice receiving normal rabbit serum (NRS) was included as the control for mice receiving the anti-IL-18 antiserum. Two hours after the fourth injection of IL-12, blood was collected from the retro-orbital plexus under methoxyflurane anesthesia. Mice were sacrificed by cervical dislocation, and the spleens and livers were removed. Spleens and livers were weighed, homogenized in 4 volumes of ice-cold PBS containing 0.2% Tween-20, and centrifuged for 5 minutes at 3,000 *g* in a microfuge. Then the supernatants were collected for cytokine measurements.

Isolation and culture of spleen cells. Spleens were aseptically removed, and cell suspensions were prepared according to standard procedures (23). Cells were washed twice in RPMI, resuspended in RPMI containing 10% FBS, and cultured at 5×10^6 /mL in 24-well plates. Cultures were incubated for various amounts of time at 37°C in a humidified atmosphere with 5% CO₂. At the end of the incubation period, supernatants were collected. The cells were resuspended in the original volume of RPMI and lysed by addition of 0.5% Triton X-100. Both supernatants and cell lysates were frozen at -70°C until they were ready for cytokine measurement.

Cytokine measurements. Both IL-18 and TNF- α levels were measured using an electrochemiluminescence

(ECL) method (24). The TNF- α assay has been previously described (25). For IL-18 measurement, a similar procedure was employed. An affinity-purified rabbit anti-murine IL-18 polyclonal antibody (R&D Systems Inc., Minneapolis, Minnesota, USA) was labeled with biotin (IGEN Inc., Gaithersburg, Maryland, USA), and a monoclonal anti-murine IL-18 antibody (R&D Systems Inc.) was labeled with ruthenium (II) trisbipyridae chelate (IGEN Inc.). The biotinylated antibody was diluted to 0.5 μ g/mL in PBS (pH 7.4) with 0.25% BSA, 0.5% Tween-20, and 0.01% sodium azide (ECL buffer). Twenty-five microliters of this antibody was combined in 6-mL polypropylene tubes with 25 μ L of a 1 mg/mL suspension of streptavidin-coated paramagnetic beads (DynaL Inc., Lake Success, New York, USA) diluted in ECL buffer. Tubes were shaken for 15 minutes at room temperature, after which 25 μ L of samples or standard concentrations of recombinant murine IL-18 (Pepro-Tech Inc., Rocky Hill, New Jersey, USA) was added. Twenty-five microliters of ruthenylated antibody (1 μ g/mL in ECL buffer) was then added, and the tubes were shaken overnight at room temperature. The reaction was quenched by addition of 200 μ L/tube of PBS and was quantitated using the Origen 1.5 Analyzer (IGEN Inc.). The ECL IL-18 assay detects both recombinant murine pro-IL-18 and mature IL-18. The range of detection is 20 pg/mL to 100 ng/mL. A 100 ng/mL concentration of pro-IL-18 is detected in the assay as 20 ng/mL of mature IL-18. The presence of mouse serum or FBS does not interfere with the detection of IL-18. IFN- γ and IL-1 β were measured using specific ELISAs (Endogen Inc.).

Western blot analysis. Spleen and livers were weighed, homogenized in 4 volumes of ice-cold PBS containing 0.2% Tween-20, and microfuged for 5 minutes at 3,000 *g*. Then the supernatants were collected for Western blot analysis. SDS-PAGE was performed on 15% gels (Bio-Rad Laboratories Inc., Hercules, California, USA); 5 μ L of samples was loaded per lane. Proteins were transferred onto nitrocellulose membranes (Amersham International, Amersham, United Kingdom) using a semidry technique. The transfer buffer contained 25 mM Tris-HCl, 192 mM glycine, 15% methanol, and 0.1% SDS. After transfer, nonspecific sites on the membranes were

Figure 1

Effect of IL-18 neutralization on the induction of IFN- γ by IL-12 in vitro. Splenocytes were cultured for 48 hours with increasing concentrations of IL-12 (0.1–10 ng/mL) in the presence or absence of either neutralizing anti-IL-18 IgG or control IgG (50 μ g/mL). IFN- γ levels were measured in the supernatants. Data are mean \pm SEM of 3 mice per group and are representative of 1 experiment out of 3 performed. **P* < 0.05, ***P* < 0.01 vs. RPMI or control IgG by repeated-measures ANOVA.

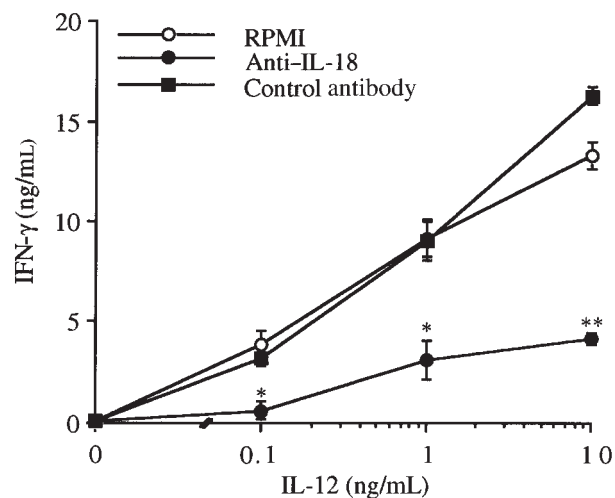
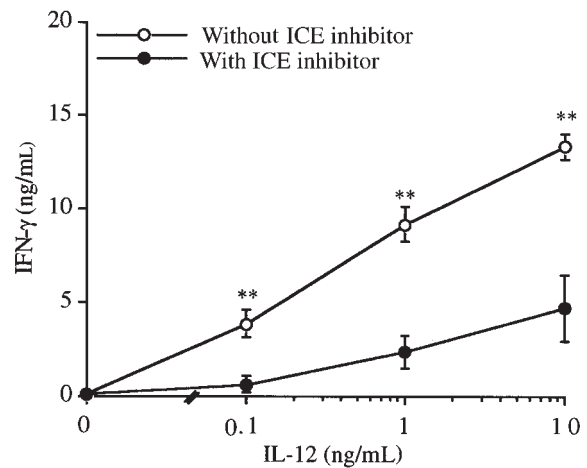


Figure 2

Effect of ICE inhibition on IFN- γ induced by IL-12 in vitro. Splenocytes were cultured for 48 hours with increasing concentrations of IL-12 (0.1–10 ng/mL) in the presence or absence of ICE inhibitor (20 μ M). IFN- γ levels were measured in the supernatants. Data are mean \pm SEM of 3 mice per group and are representative of 1 experiment out of 3 performed. * P < 0.05, ** P < 0.01 by paired Student's t test.



blocked overnight at 4°C with PBS containing 5% non-fat milk, 1% BSA, and 0.1% Tween-20. Blots were probed with a rabbit anti-murine IL-18 antiserum (26), diluted at 1:400, for 1 hour at room temperature, followed by 3 washes in PBS containing 0.1% Tween-20. A secondary horseradish peroxidase-conjugated antibody (1:6,000 dilution; The Jackson Laboratory, Bar Harbor, Maine, USA) was added for 45 minutes at room temperature. The blot was washed 3 times in PBS/Tween-20, and the antibody was detected by ECL (NEN Life Science Products, Boston, Massachusetts, USA).

Results

Neutralization of IL-18 inhibits induction of IFN- γ by IL-12 in murine splenocytes. Splenocytes were incubated for 48 hours with increasing concentrations of IL-12 in the presence or absence of neutralizing anti-IL-18 IgG (50 μ g/mL) (13). As shown in Figure 1, a concentration-dependent increase of IFN- γ production was observed in cells incubated with IL-12 alone. Neutralization of IL-18 significantly reduced IFN- γ levels at each of the concentrations of IL-12 tested. On the other hand, when cells were incubated with IL-12 in the presence of an unrelat-

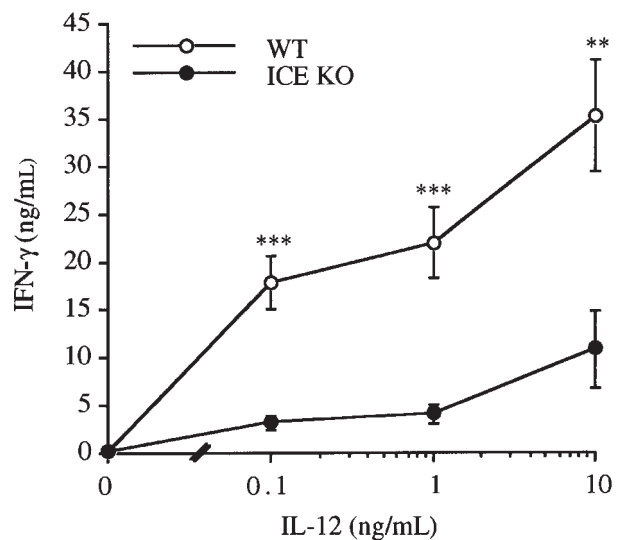
ed IgG (anti-CSF), no decrease in IFN- γ levels was observed. The inhibitory effect of anti-IL-18 IgG was specific for IFN- γ . In fact, in the same samples, neutralization of IL-18 did not affect IL-12-induced TNF- α levels (data not shown).

Inhibition of ICE activity or ICE deficiency reduces IL-12-induced IFN- γ production. Splenocytes were incubated for 48 hours with increasing concentrations of IL-12 in the presence or absence of a specific ICE inhibitor (27). As shown in Figure 2, inhibition of ICE activity significantly reduced production of IFN- γ at each of the concentrations of IL-12. As already observed with anti-IL-18 IgG, the ICE inhibitor did not reduce IL-12-induced TNF- α levels (data not shown).

To test the effect of ICE deficiency on IL-12-induced IFN- γ production, splenocytes obtained from WT and ICE KO mice were cultured for 48 hours with increasing concentrations of IL-12. As shown in Figure 3, significantly lower levels of IFN- γ were observed in cells obtained from ICE KO mice compared with cells derived from WT mice. No differences in IL-12-induced TNF- α levels were observed between WT and ICE KO splenocytes (data not shown).

Figure 3

Production of IFN- γ in splenocytes from ICE KO mice. Splenocytes from WT or ICE KO mice were cultured for 48 hours with increasing concentrations of IL-12 (0.1–10 ng/mL). IFN- γ levels were measured in the supernatants. Data are mean \pm SEM of 6 mice per group. ** P < 0.01, *** P < 0.001 WT vs. ICE KO by unpaired Student's t test.



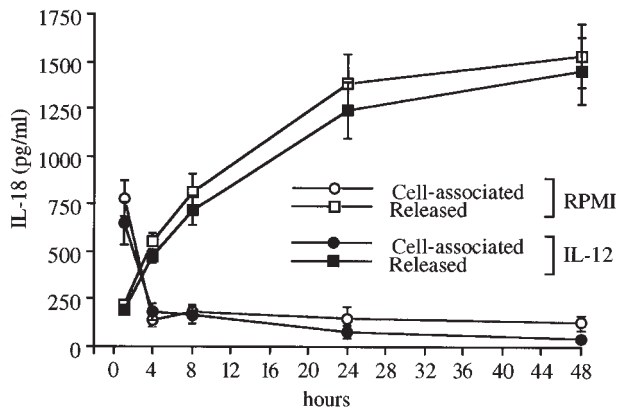


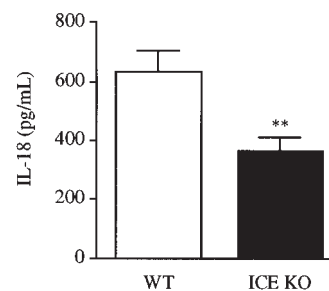
Figure 4 IL-18 production in unstimulated and IL-12-stimulated splenocytes. Splenocytes were cultured in RPMI-FBS for increasing amounts of time without added exogenous stimulation, or with IL-12 at 10 ng/mL. Cell-associated and released IL-18 levels were measured. Data are mean \pm SEM of 9 mice per group.

Blockade of IL-1 receptor does not affect IL-12-induced IFN- γ production. Inhibition of ICE activity leads to reduced levels of both mature IL-18 and mature IL-1 β (8, 28). However, blocking IL-1 receptors by using saturating concentrations of IL-1Ra did not affect induction of IFN- γ by IL-12 in cultures of murine splenocytes (after stimulation with 10 ng/mL of IL-12, IFN- γ levels were 12.3 ± 0.8 vs. 10.9 ± 1.2 ng/mL in the absence or presence of IL-1Ra, respectively; $n = 3$). Furthermore, IL-1 β was not detectable (<10 pg/mL) in control or IL-12-stimulated splenocytes obtained from WT mice (data not shown). We can therefore conclude that, in this system, IL-1 β does not play a significant role in the induction of IFN- γ by IL-12.

IL-18 production by cultured splenocytes. To evaluate whether IL-12 induces IL-18 production in vitro, splenocytes were cultured for increasing amounts of time in the presence or absence of IL-12. IL-18 levels were measured separately in the supernatants and in the cell lysates.

As shown in Figure 4, high levels of IL-18 were present in the cell lysates of unstimulated splenocytes cultured for 1 hour (782.00 ± 97.30 pg/mL; $n = 9$). These intracellular levels of IL-18 were not significantly different from those observed in lysates obtained immediately after isolation of splenocytes, i.e., without culturing the cells (732.34 ± 54.36 pg/mL; $n = 9$). As shown in Figure 4, a decrease in the cell-associated concentration of IL-18 was observed as the time of incubation increased.

Figure 5 Release of IL-18 in splenocytes from ICE KO mice. Splenocytes from WT or ICE KO mice were cultured for 24 hours in the absence of any exogenous stimulation. IL-18 levels were measured in supernatants. Data are mean \pm SEM of 8 mice per group. ** $P < 0.01$ by factorial ANOVA.



Low levels of IL-18 were present in the supernatants of cells cultured for 1 hour. However, a greater release of IL-18 into the extracellular compartment occurred over time. TNF- α was not detectable in cell lysates or supernatants of unstimulated splenocytes at any of the time points tested.

In cells stimulated with IL-12, the amount of intracellular and extracellular IL-18 did not differ from that of unstimulated splenocytes (Figure 4). On the other hand, in the same IL-12-stimulated samples, high levels of IFN- γ and moderately increased levels of TNF- α were observed after 24 or 48 hours in culture (data not shown).

A positive correlation was observed between supernatant levels of IL-18 and IFN- γ production ($r^2 = 0.786$, $P = 0.014$; $n = 9$). On the other hand, there was no significant correlation between IL-18 and TNF- α levels (data not shown).

Reduced release of IL-18 in ICE KO mice. To verify whether splenocytes from ICE KO mice have a defective IL-18 release, spleen cells from WT and ICE KO mice were cultured for 24 hours in the absence of any exogenous stimulation. As shown in Figure 5, significantly lower levels of IL-18 were present in the supernatants of ICE KO splenocytes compared with WT cells.

Reduced serum IFN- γ levels in ICE KO mice after in vivo administration of IL-12. As shown in Figure 6, after daily administration of IL-12 (100 ng/mouse for 4 days), significantly lower levels of IFN- γ were present in the serum of ICE KO mice compared with WT mice. A similar observation was made when IL-12 was administered at 400 ng/mouse per day. However, with this higher dose of IL-12, the difference between ICE KO and WT mice was no longer statistically significant.

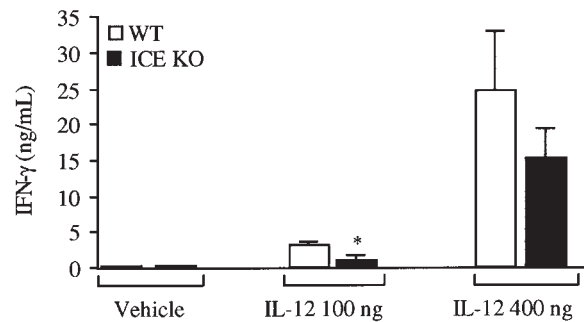
The effect of ICE deficiency is specific for IFN- γ induction, because IL-12-induced serum TNF- α levels did not differ between WT and ICE KO mice (162.35 ± 4.97 vs. 171.51 ± 14.76 pg/mL in WT and ICE KO mice, respectively, after 4 daily injections of IL-12 at 100 ng/mouse per day; $n = 5$).

IL-12 administration increases serum and liver-associated IL-18 levels. Consistent with the low IFN- γ levels observed in ICE KO mice, administration of IL-12 at 100 or 400 ng/mouse per day induced a significant increase in circulating IL-18 levels in WT mice but not in ICE KO mice (Figure 7). Measurement of IL-18 in serum versus EDTA or heparin plasma leads to comparable results (data not shown).

High levels of IL-18 were present in the freshly prepared liver homogenates of untreated, healthy mice (29.05 ± 5.6 and 28.55 ± 6.14 ng/g in WT and ICE KO

Figure 6

Effect of in vivo administration of IL-12 on IFN- γ levels in ICE KO and WT mice. WT and ICE KO mice received 4 daily intraperitoneal injections of IL-12 (100 or 400 ng/mouse) or vehicle. Two hours after the fourth injection, blood was collected and serum was prepared for measurement of IFN- γ . Data are mean \pm SEM of 10 mice per group. * $P < 0.05$ vs. WT mice by factorial ANOVA.



mice, respectively; $n = 10$). The presence of pro-IL-18 in the livers of untreated mice was confirmed by Western blot analysis (Figure 8). Administration of IL-12 at 400 ng/mouse per day significantly increased liver-associated IL-18 in both WT and ICE KO mice (112.79 ± 16.42 and 105.88 ± 38.13 ng/g in WT and ICE KO mice, respectively; $n = 10$). Contrary to what was observed in the serum, there were no significant differences between ICE KO mice and WT mice for liver-associated IL-18 production.

The IL-18 assay also detected high levels of IL-18 in the spleen homogenates of untreated mice. The presence of pro-IL-18 in the spleens of untreated mice was confirmed by Western blot analysis (Figure 8). IL-12 administration did not significantly alter spleen-associated IL-18 levels when expressed as nanograms per gram of tissue (data not shown). However, because IL-12 administration induced a marked splenomegaly (3), the amount of IL-18 produced per spleen was significantly higher in animals treated with 400 ng/d of IL-12 (30.53 ng IL-18/spleen) compared with the control group (6.35 ng/spleen; $n = 5$; $P < 0.001$ by unpaired Student's t test). No significant differences in spleen IL-18 content were observed between WT and ICE KO mice.

TNF- α was below detectable limits in both the liver and the spleen homogenates of untreated mice, therefore demonstrating that the high levels of IL-18 observed in these organs were unlikely to be due to the presence of underlying disease in the mice. In addition, as reported previously (5), IL-12 administration increased spleen-associated TNF- α levels (0.20 ± 0.05 and 0.22 ng/g in WT and ICE KO mice, respectively, after 4 daily injections of IL-12 at 400 ng/mouse per day; $n = 5$).

Neutralization of IL-18 reduces IFN- γ production in vivo. The results suggest that IL-18 significantly contributes to

induction of IFN- γ after in vivo administration of IL-12. To further confirm these results, a neutralizing rabbit anti-IL-18 antiserum was administered before the first and third injection of IL-12 (100 ng/mouse per day). IFN- γ levels were measured in the serum 2 hours after the fourth injection. The control group received the same amount of NRS. As shown in Figure 9, neutralization of IL-18 reduced IL-12-induced IFN- γ production significantly.

Discussion

In the present report, we have shown that endogenous IL-18 participates in the induction of IFN- γ by IL-12 in mice, both in vitro and in vivo. The role of endogenous IL-18 in the regulation of IFN- γ by microbial stimuli such as endotoxin, zymosan, or live bacteria has been demonstrated previously (13, 29). The novelty of the present report is the demonstration that neutralization of endogenous IL-18 significantly reduces production of IFN- γ when induced directly by IL-12, not by a microbial stimulus.

Our results imply that endogenous IL-18 plays an important and significant role in the induction of IFN- γ by IL-12. Similarly, IL-12-induced NK-cell activity requires endogenous IL-18, because lower cytotoxicity is observed in cells obtained from IL-18 KO mice (11). However, induction of both IFN- γ and NK-cell activity by IL-12 are not completely IL-18-dependent, especially when high doses of IL-12 are employed (ref. 11 and this paper).

Inhibition of ICE activity or ICE deficiency reduced induction of IFN- γ by IL-12, both in vitro and in vivo. The role of ICE in regulating IFN- γ production was through IL-18, not IL-1 β processing. In fact, IFN- γ production in response to IL-12 was not affected by blockade of IL-1 receptors. Moreover, IL-12 did not induce

Figure 7

Increase in serum IL-18 levels after IL-12 administration. WT and ICE KO mice received 4 daily intraperitoneal injections of IL-12 (100 or 400 ng/mouse) or vehicle. Two hours after the fourth injection, blood was collected and serum was prepared. Data are mean \pm SEM of 10 mice per group. * $P < 0.05$ vs. WT mice. † $P < 0.01$ vs. corresponding vehicle by factorial ANOVA.

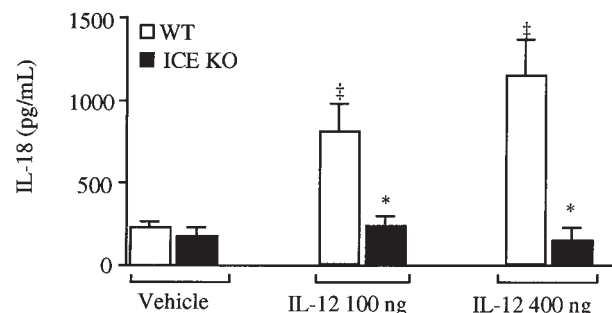
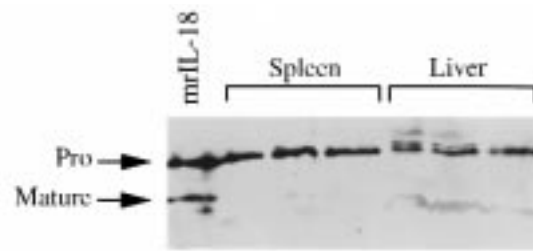


Figure 8

Constitutive expression of pro-IL-18 in the spleens and livers on untreated mice. Spleen and liver homogenates from 3 untreated mice were subjected to Western blot analysis with a specific rabbit anti-murine IL-18 antiserum. Recombinant murine pro-IL-18 and mature IL-18 were used as standards.



IL-1 β production.

A newly developed assay for detection of IL-18 was used in the present study to evaluate the production of this cytokine in response to IL-12. Compared with other proinflammatory cytokines, IL-18 is regulated in a unique way. In fact, constitutive levels of IL-18 mRNA and protein are present in unstimulated human and murine cells and in the tissues of untreated, healthy mice (refs. 20, 26, 30–33, and this paper). These data are consistent with the finding that one of the two IL-18 promoters is constitutively active (30). In unstimulated cells or tissues, IL-18 is present primarily in the precursor form, but requires conversion into the bioactive, mature molecule (26, 32). We observed constitutive intracellular levels of precursor IL-18 in freshly isolated spleen cells. Incubation of the cells at 37°C for 8–48 hours resulted in the release of IL-18 into the extracellular compartment in the absence of any exogenous stimulation. Although the assay used in this study for IL-18 determination detects both the precursor and mature forms, IL-18 found in the extracellular compartment is mostly in the mature form. Two sets of data support this concept: (a) reduced levels of extracellular IL-18 were observed in cultures of ICE KO splenocytes; and (b) a significant correlation was observed between extracellular IL-18 levels and IL-12-induced IFN- γ production. In vitro, IL-18 is constitutively present and is available to act with a second stimulus, in this case IL-12. IL-18 alone is a weak inducer of IFN- γ production, possibly owing to a low constitutive expression of IL-18 receptors on T cells (17). Therefore, it is not surprising that, even in the presence of mature, bioactive IL-18, no IFN- γ is detectable in unstimulated spleen cell cultures; a second stimulus, such as IL-12, is necessary for induction of IFN- γ .

Extracellular levels of IL-18 were significantly reduced,

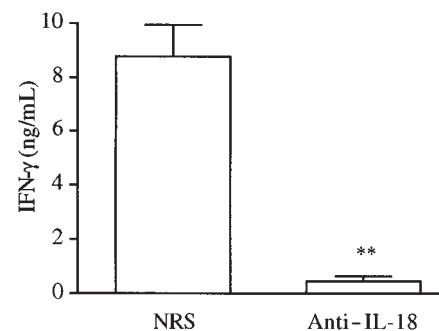
but not absent, in cultures of ICE KO spleen cells. Two explanations are possible for this phenomenon: (a) ICE is not the only enzyme responsible for the cleavage and release of IL-18 from the intracellular compartment; and (b) when ICE is not present or its activity is inhibited, IL-18 can be released from the cell in the precursor form. Both mechanisms have been demonstrated for cleavage and release of IL-1 β (34–37).

An increase in circulating IL-18 levels was observed in vivo in IL-12-injected mice. This observation is in agreement with results obtained by Lauw et al. in IL-12-injected chimpanzees (21). Compared with WT mice, significantly lower levels of serum IFN- γ were observed in ICE KO mice after administration of IL-12 at 100 ng/mouse per day. Consistently, circulating IL-18 levels were low in ICE KO mice compared with WT mice, again demonstrating the important role of ICE in the cleavage and release of IL-18. Circulating IL-18 levels remained low in ICE KO mice even when high doses of IL-12 were administered. In this case, IL-12-induced IFN- γ production was relatively independent of IL-18, because ICE KO mice had low IL-18 but high IFN- γ levels in their circulation. Similar results were obtained previously by evaluating IL-12-induced NK-cell cytotoxicity in IL-18 KO mice. Also, in that case, the effect of IL-12 was IL-18-dependent only when lower doses of IL-12 were used (11).

Administration of IL-12, particularly at the higher dose, also significantly increased liver-associated IL-18 levels. The Kupffer cell is the most likely source for the hepatic production of IL-18 (14). Consistent with observations that tissue-associated IL-18 is mostly present in the precursor form (26), no differences in the liver IL-18 content were observed between WT and ICE KO mice. As reported previously (3), administration of IL-12 induced hepatomegaly and splenomegaly. No differences in these parameters were observed between WT

Figure 9

Neutralization of IL-18 reduces IL-12-induced IFN- γ levels in vivo. Mice received 4 daily intraperitoneal injections of IL-12 (100 ng/mouse). One hour before the first and the third injection, the antibody-treated group received an intraperitoneal injection of 200 μ L of rabbit anti-IL-18 antiserum, whereas the control group received the same amount of NRS. Two hours after the fourth injection, blood was collected and serum was prepared for measurement of IFN- γ . Data are mean \pm SEM of 5 mice per group. ** P < 0.01 vs. control by unpaired Student's t test.



and ICE KO mice (data not shown). As a consequence of the increase in organ weight, the total content of IL-18 in the liver, and particularly the spleen, was highly increased after administration of IL-12.

In conclusion, the present report demonstrates that ICE-cleaved endogenous IL-18 significantly contributes to induction of IFN- γ by IL-12, both in vitro in cultures of murine splenocytes and in vivo in mice chronically injected with IL-12. Although IL-12 administration does increase IL-18 levels, this is not strictly necessary, because IL-18 is constitutively produced in liver and spleen cells. It is possible that the constitutively expressed IL-18 binding protein (38) may serve as a natural mechanism for binding constitutive IL-18 and controlling its bioactivity.

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