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Caspase-1-Independent, Fas/Fas Ligand–Mediated IL-18 Secretion from Macrophages Causes Acute Liver Injury in Mice

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Summary

IL-18, produced as a biologically inactive precursor, is processed by caspase-1 in LPS-activated macrophages. Here, we investigated caspase-1-independent processing of IL-18 in Fas ligand (FasL)-stimulated macrophages and its involvement in liver injury. Administration of *Propionibacterium acnes* (*P. acnes*) upregulated functional Fas expression on macrophages in an IFNγ-dependent manner, and these macrophages became competent to secrete mature IL-18 upon stimulation with FasL. This was also the case for caspase-1-deficient mice. Administration of recombinant soluble FasL (rFasL) after *P. acnes* priming induced comparable elevation of serum IL-18 in parallel with elevated serum liver enzyme levels. However, liver injury was not induced in IL-18-deficient mice after rFasL administration. These results indicate a caspase-1-independent pathway of IL-18 secretion from FasL-stimulated macrophages and its critical involvement in FasL-induced liver injury.

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Introduction

IL-18 is a potent pleiotropic cytokine (Okamura et al., 1995, 1998a, 1998b; Dinarello et al., 1998). IL-18 induces IFNγ production by lymphocytes, such as T cells and NK cells, particularly in a synergistic manner with IL-12 (Okamura et al., 1995; Tsutsui et al., 1997; Yoshimoto et al., 1998). IL-18 augments NK activity through the activation of constitutively expressed IL-18R on NK cells (Hyodo et al., 1999). Moreover, IL-18 upregulates functional Fas ligand (FasL) expression on NK cells and T cells (Dao et al., 1996; Tsutsui et al., 1996).

IL-18 has a structural homology to IL-1, particularly IL-1β, although its functions differ from those of IL-1 (Bazan et al., 1996; Dinarello et al., 1998; Okamura et al., 1998a, 1998b). IL-18R, composed of IL-1R related protein (Torigoe et al., 1997) and accessory protein-like (Born et al., 1998), belongs to the IL-1R family. Like IL-1β, IL-18 requires intracellular processing for its secretion. IL-18 is intracellularly produced as a biologically inactive precursor (proIL-18), and mature IL-18 is secreted after the cleavage of proIL-18 by caspase-1, originally designated as IL-1β converting enzyme (ICE) (Ghayur et al., 1997; Gu et al., 1997). Kupffer cells from caspase-1-deficient mice did not secrete IL-18 after LPS stimulation, whereas those from wild-type C57BL/6 mice did (Gu et al., 1997). LPS challenge induced IL-18 in the serum of *Propionibacterium acnes* (*P. acnes*)-primed wild-type C57BL/6 mice, while IL-18 was not observed in the serum of *P. acnes*-primed caspase-1-deficient mice (Gu et al., 1997). This sequential treatment with heat-killed *P. acnes* and LPS induces IL-18-dependent acute liver injury in mice (Tsutsui et al., 1992, 1997; Okamura et al., 1995). IL-18 secreted by LPS-activated *P. acnes*-elicited Kupffer cells initiates the activation of hepatotoxic cytokine network in the liver, including FasL expression (Tsutsui et al., 1997). The treatment with neutralizing anti-IL-18 antibody protects mice from the liver injury (Okamura et al., 1995), and IL-18-deficient mice are resistant to the liver injury (Sakao et al., 1999), indicating that IL-18 plays a critical role in this liver injury. Recently, it has been reported that mature IL-1β was secreted from neutrophils after FasL stimulation in the absence of caspase-1 (Miwa et al., 1998). This prompted us to explore the possibility that the secretion of mature IL-18 might also be induced by FasL and that the FasL-induced liver injury might be caused by IL-18 secreted from FasL-stimulated macrophages. Here, we investigated whether IL-18 is processed by the stimulation with FasL. Biologically active IL-18 was secreted from *P. acnes*-elicited Fas-expressing macrophages after the stimulation with FasL. This was also the case for *P. acnes*-elicited macrophages from caspase-1-deficient mice. The FasL-induced secretion of IL-18 was inhibited by caspase inhibitors that prevented the macrophages from apoptosis, suggesting that some caspase other than caspase-1 is involved in the processing of IL-18 in the FasL-stimulated macrophages. In addition, the administration of recombinant human soluble FasL (rFasL) induced elevated serum levels of IL-18 in *P.*

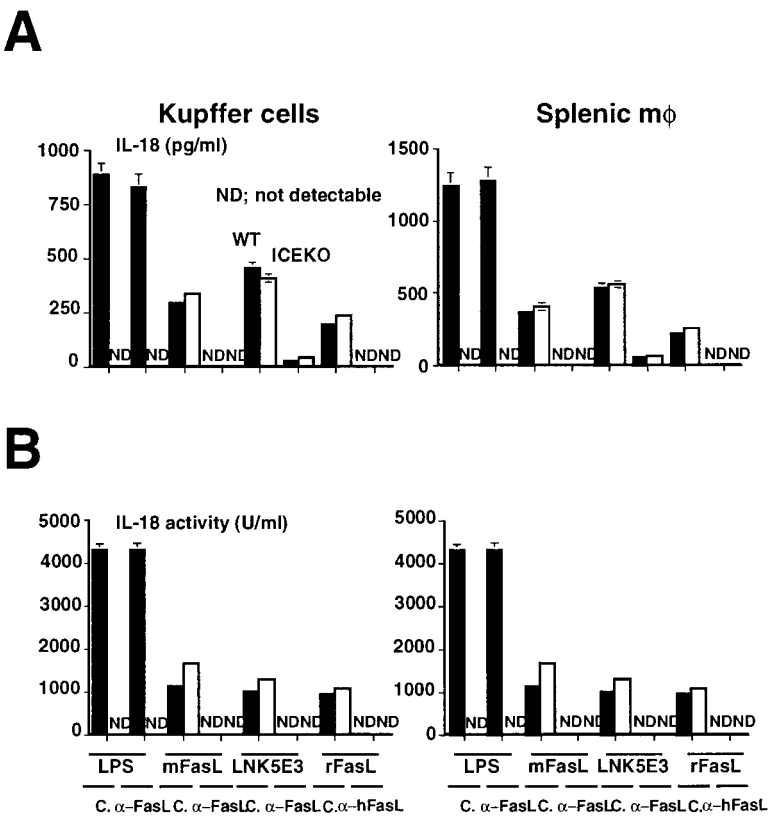

Figure 1. Mature IL-18 Secretion from *P. acnes*-Elicited Macrophages by Stimulation with FasL

(A) IL-18 secretion by FasL-stimulated macrophages. Kupffer cells (left) or splenic macrophages (splenic mø, right) (1×10^6/ml) were prepared from *P. acnes*-primed C57BL/6 mice (WT, closed column) or caspase-1-deficient mice (ICEKO, open column). The cells were incubated with 1 μg/ml of LPS, 2×10^6/ml of mFasL cells, or 2×10^6/ml of LNK5E3 cells in the presence of 10 μg/ml anti-mouse FasL mAb (a-FasL) or control hamster IgG (C) for 24 hr. The cells were also incubated with 20 ng/ml of rFasL in the presence of 50 μg/ml of anti-human FasL mAb (α-hFasL) or control isotype-matched mouse IgG (C) for 24 hr. IL-18 in the supernatant was measured by ELISA. Data are indicated as mean ± SD of triplicated cultures and represent one of three experiments with similar results. ND, not detectable. (B) Secretion of biologically active IL-18 from FasL-stimulated macrophages. LNK5E3 cells (1×10^6/ml) were incubated with each supernatant shown in (A) in the presence or absence of 100 μg/ml of neutralizing anti-IL-18 antibody and 100 pg/ml of IL-12 for 48 hr. Concentration of IFNγ in each supernatant was determined by ELISA. IL-18 activity was calculated as described in the Experimental Procedures. Data are indicated as mean ± SD of triplicated cultures and are representative of three independent experiments with similar results. ND, not detectable.

acnes-primed caspase-1-deficient mice as well as wild-type C57BL/6 mice, both leading to acute liver injury. In contrast, the same treatment did not cause liver injury in *P. acnes*-primed IL-18-deficient mice, indicating the critical role of IL-18 in the FasL-induced liver injury.

Results

Caspase-1-Independent IL-18 Secretion from FasL-Stimulated Macrophages

To investigate whether IL-18 can be secreted from FasL-stimulated macrophages in a caspase-1-independent manner, we incubated Kupffer cells or splenic macrophages from *P. acnes*-primed wild-type C57BL/6 mice or caspase-1-deficient mice (presented as ICEKO in the following figures) with murine FasL-transfected L5178Y (mFasL) cells, constitutively FasL-expressing cloned hepatic NK (LNK5E3) cells, or rFasL. As shown in Figure 1A, left, *P. acnes*-elicited Kupffer cells from wild-type and caspase-1-deficient mice secreted comparable levels of IL-18 after stimulation with FasL transfectants or LNK5E3 cells as determined by ELISA. This secretion of IL-18 was inhibited by neutralizing mAb against murine FasL, indicating that IL-18 secretion from *P. acnes*-elicited Kupffer cells was induced by murine FasL expressed on those cells. IL-18 secretion was also induced by rFasL, indicating that FasL can solely induce IL-18 secretion from *P. acnes*-primed Kupffer cells in a caspase-1-independent manner (Figure 1A). In contrast, LPS stimulation did not induce IL-18 secretion from the Kupffer cells from *P. acnes*-primed caspase-1-deficient mice, indicating that IL-18 release from LPS-stimulated macrophages totally depends on caspase-1, as demonstrated previously (Gu et al., 1997). We also demonstrated FasL-stimulated IL-18 secretion from *P. acnes*-elicited splenic macrophages. As shown in Figure 1A, right, comparable levels of IL-18 were also detected in the supernatant of *P. acnes*-elicited splenic macrophages from wild-type C57BL/6 mice and caspase-1-deficient mice upon stimulation by mFasL cells, LNK5E3 cells, or rFasL. The prerequisite of caspase-1 for LPS-stimulated secretion of IL-18 was also the case for *P. acnes*-elicited splenic macrophages. Both Kupffer cells and splenic macrophages from nontreated wild-type or caspase-1-deficient mice did not release IL-18 after the stimulation with FasL (data not shown), suggesting that the *P. acnes* priming is a prerequisite for making macrophages competent to respond to the FasL-stimulated IL-18 secretion.

In order to examine biological activity of IL-18 secreted from the FasL-stimulated Kupffer cells or macrophages, we measured IFNγ production by LNK5E3 cells incubated with the supernatants from FasL-stimulated cells. As shown in Figure 1B, left, the supernatant of FasL-stimulated *P. acnes*-elicited Kupffer cells from either wild-type C57BL/6 mice or caspase-1-deficient mice induced IFNγ production by LNK5E3 cells, indicating that IL-18 secreted by these cells had biological activity. Similar results were obtained with the FasL-stimulated splenic macrophages (Figure 1B, right). The biological activities in the supernatants from LPS- or FasL-stimulated macrophages were well correlated with

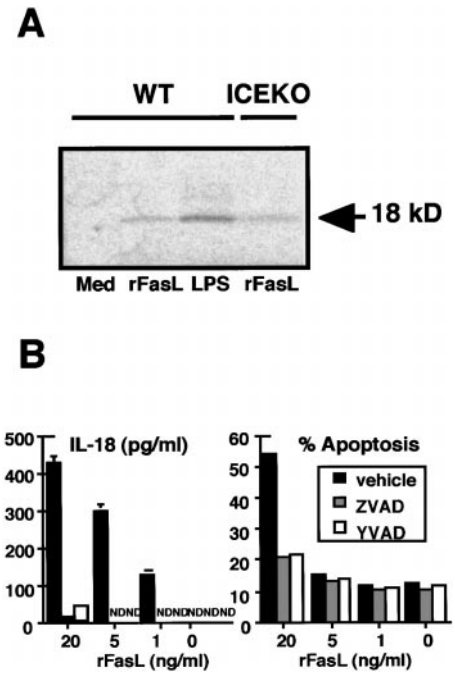

Figure 2. Caspase-Dependent IL-18 Secretion from FasL-Stimulated *P. acnes*-Elicited Macrophages

(A) Secretion of 18 kDa mature IL-18 from rFasL-stimulated *P. acnes*-elicited Kupffer cells. *P. acnes*-elicited Kupffer cells from wild-type C57BL/6 mice (WT) or caspase-1-deficient mice (ICEKO) were incubated with [^{35}S]-methionine/cysteine in the presence or absence of 1 μg/ml LPS or 20 ng/ml of rFasL for 5 hr. The resulting supernatant was immunoprecipitated using protein G-Sepharose and anti-IL-18 antibody. Metabolically labeled IL-18 in each supernatant was determined by SDS-PAGE and autoradiography. Arrow indicates 18 kDa mature IL-18.

(B) Inhibitory effect of caspase inhibitors on IL-18 secretion from rFasL-stimulated macrophages. *P. acnes*-elicited splenic macrophages from C57BL/6 mice were incubated with various doses of rFasL in the presence of 20 μM Z-VAD-FMK (ZVAD), 20 μM Ac-YVAD-CMK (YVAD), or the same volume of DMSO (vehicle) for 24 hr. IL-18 in each supernatant was measured by ELISA (left). The cells were analyzed for apoptosis by the pattern of nuclear staining with propiodium iodide (right). Data are indicated as mean ± SD of triplicated cultures and representative of three independent experiments with similar results. ND, not detectable.

the IL-18 levels determined by ELISA (Figure 1A), suggesting that the IL-18 secreted from the FasL-stimulated Kupffer cells or macrophages in a caspase-1-independent manner has equivalent activity to the conventional IL-18 secreted from the LPS-stimulated macrophages in a caspase-1-dependent manner.

Abrogation of Fas-Mediated IL-18 Secretion by Caspase Inhibitors

To investigate whether the FasL-stimulated IL-18 secretion from *P. acnes*-elicited Kupffer cells is associated with the processing of IL-18, as observed in the case of LPS-stimulated *P. acnes*-elicited Kupffer cells (Gu et al., 1997), we performed immunoprecipitation of metabolically labeled IL-18 from the supernatant of rFasL- or LPS-stimulated Kupffer cells. As shown in Figure 2A, an 18 kDa form of IL-18 was detected in the supernatant of rFasL-stimulated *P. acnes*-elicited Kupffer cells from either caspase-1-deficient or wild-type C57BL/6 mice, which was just the same size as the mature IL-18 found in LPS-activated Kupffer cell supernatant (Gu et al., 1997). Therefore, IL-18 can be processed at a site close to the cleavage site for caspase-1 in FasL-stimulated Kupffer cells independently of caspase-1.

In order to test involvement of caspases other than caspase-1, we incubated the macrophages with general inhibitors for caspase. As shown in Figure 2B, both Z-VAD-FMK (ZVAD) and Ac-YVAD-CMK (YVAD) inhibited IL-18 secretion from the macrophages as well as their apoptosis. Therefore, some caspase other than caspase-1 is involved in the processing of proIL-18 in FasL-stimulated macrophages. It was also noted that IL-18 secretion from FasL-stimulated macrophages was not strictly correlated with their apoptosis as observed at 5 ng/ml of rFasL (Figure 2B). Similar results were obtained from *P. acnes*-elicited macrophages from caspase-1-deficient mice (data not shown).

Fas Expression on *P. acnes*-Elicited Macrophages

To investigate a mechanism of *P. acnes*-induced sensitization to FasL, we examined Fas expression on macrophages before and after the treatment with *P. acnes*. As shown in Figure 3, Mac1-positive Kupffer cells from both wild-type and caspase-1-deficient mice expressed Fas at high levels after in vivo administration of *P. acnes*, while those from nontreated mice did not express Fas. In accordance with Fas expression, IL-18 was not detected in their supernatants when Kupffer cells or splenic macrophages from nontreated mice were incubated with mFasL or rFasL (data not shown). These results suggest that *P. acnes* priming is required for sensitizing macrophages to FasL-stimulated IL-18 secretion by upregulating Fas expression on these cells.

As IFNγ has been reported to upregulate Fas expression on macrophages in vitro (Spanaus et al., 1998), we next examined the Fas expression on Kupffer cells from IFNγ-deficient mice before and after the treatment with *P. acnes*. As shown in Figure 3, *P. acnes* treatment did not induce Fas expression on the IFNγ-deficient macrophages. Similar results were obtained for splenic macrophages (data not shown). Interestingly, IL-18-deficient macrophages were able to induce Fas expression after *P. acnes* treatment, suggesting that IFNγ induced by other cytokines, such as IL-12 (Matsui et al., 1997), contributes to this process. These results indicate that IFNγ plays an essential role in *P. acnes*-induced upregulation of Fas expression on macrophages.

Induction of IL-18-Dependent Acute Liver Injury by In Vivo Administration of Soluble FasL

The in vitro stimulation with rFasL induced secretion of IL-18 from *P. acnes*-primed macrophages (Figure 1). To investigate the FasL-stimulated IL-18 secretion in vivo, we administered rFasL in nontreated or *P. acnes*-primed mice. As shown in Figure 4A, serum IL-18 levels were increased after administration of rFasL in *P. acnes*-primed wild-type C57BL/6 mice, while those of nontreated wild-type C57BL/6 mice remained unchanged. IL-18 secreted in the sera had IFNγ-inducing activity (data not shown). As expected from the in vitro results, *P. acnes*-treated caspase-1-deficient mice showed the

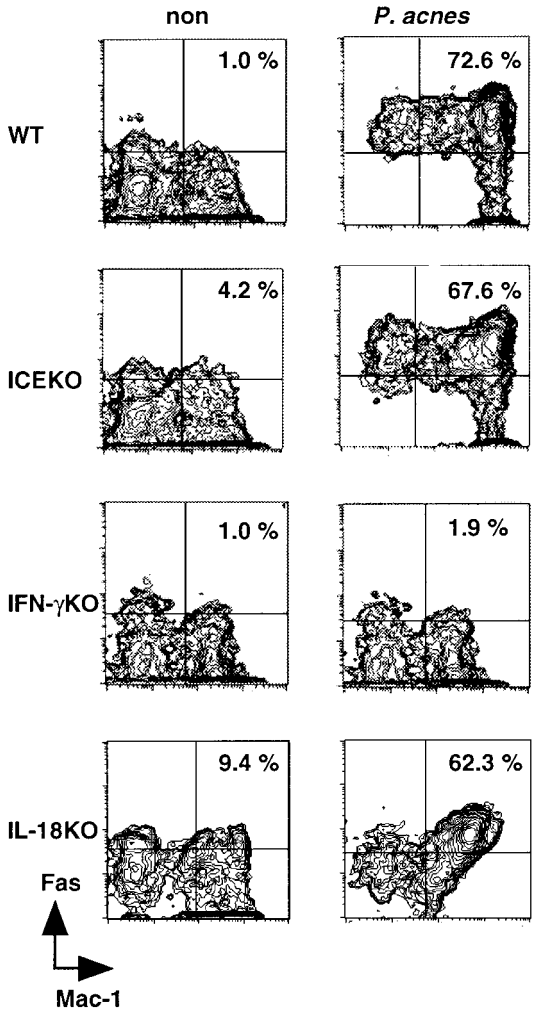

Figure 3. Fas Expression on *P. acnes*-Elicited Kupffer Cells

Kupffer cells were isolated from nontreated or *P. acnes*-primed wild-type C57BL/6 mice (WT), caspase-1-deficient mice (ICEKO), and IFNγ-deficient mice (IFNγKO). Splenic macrophages were from nontreated or *P. acnes*-primed IL-18-deficient mice (IL-18KO). The cells were stained with PE-conjugated anti-Fas and FITC-conjugated anti-Mac1 mAbs and analyzed by flow cytometry. The figure is representative of three independent experiments with similar results.

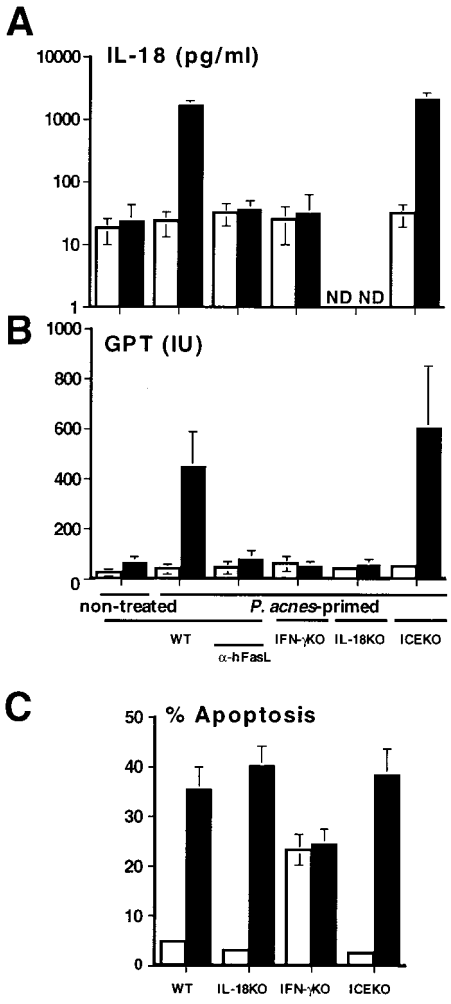

Figure 4. IL-18 Is Required for rFasL-Induced Liver Injury

(A) Elevated serum IL-18 levels after administration of rFasL into *P. acnes*-primed mice. rFasL (500 ng, closed column) or the same volume of PBS (open column) was i.v. administered into *P. acnes*-primed or nontreated wild-type C57BL/6 mice (WT), IFNγ-deficient mice (IFNγKO), IL-18-deficient mice (IL-18KO), or caspase-1-deficient mice (ICEKO). In some groups, 1 mg of neutralizing anti-human FasL mAb (α-hFasL) was i.p. administered 1 hr before. After 24 hr, serum was sampled, and IL-18 in the serum was determined by ELISA. Data represent mean ± SD of five mice in each experimental group. Similar results were obtained in three independent experiments.

(B) Elevated serum liver enzyme levels after treatment with rFasL. GPT was measured in each serum from the mice shown in (A). Data represent mean ± SD of five mice in each group. Similar results were obtained in three independent experiments.

(C) Fas-dependent apoptosis of *P. acnes*-elicited IL-18-deficient macrophages. Splenic macrophages were isolated from nontreated (open column) or *P. acnes*-primed (closed column) wild-type C57BL/6 mice (WT), IL-18-deficient mice (IL-18KO), IFNγ-deficient mice (IFNγKO), or caspase-1-deficient mice (ICEKO) and incubated with 20 ng/ml of rFasL for 48 hr followed by PI staining. Data are represented as mean ± SD of triplicated cultures. Similar results were obtained in three independent experiments.

elevated serum level of IL-18 after administration of rFasL, which was comparable to that in wild-type C57BL/6 mice. This indicates that FasL-stimulated IL-18 secretion from *P. acnes*-primed macrophages is caspase-1 independent also in vivo. In contrast, IL-18 elevation was not observed in IFNγ-deficient mice (Figure 4A), possibly due to lack of Fas expression after *P. acnes* priming (Figure 3).

Next, we examined the biological consequences of FasL-induced IL-18 secretion in *P. acnes*-primed mice. It has been reported that liver injury could not be readily induced by the administration of rFasL unless the mice had been treated with *P. acnes* (Tanaka et al., 1997). This prompted us to address the possibility that rFasL-induced IL-18 secretion might play a critical role in induction of acute liver injury, rather than direct cytotoxic action of rFasL against liver parenchymal cells. To investigate this possibility, we measured serum liver enzyme levels in *P. acnes* and rFasL-treated mice with various genomic mutations. As expected, *P. acnes*-primed wild-type C57BL/6 mice showed acute liver injury after rFasL challenge, whereas nontreated wild-type C57BL/6 mice did not suffer from liver injury. A comparable level of

liver injury was caused in caspase-1-deficient mice, indicating that caspase-1 is dispensable for this liver injury. In contrast, neither IL-18-deficient nor IFNγ-deficient mice suffered from liver injury (Figure 4B). The susceptibility of the mice to this liver injury was well correlated with the elevated serum IL-18 levels shown in Figure 4A. As shown in Figures 3 and 4C, splenic macrophages from IL-18-deficient mice expressed Fas after *P. acnes* treatment and underwent apoptosis by rFasL stimulation in vitro similarly to those from wild-type C57BL/6 mice, indicating that *P. acnes*-elicited macrophages in IL-18-deficient mice expressed functional Fas on their surface. Thus, these results indicated that IL-18 plays a critical role in induction of acute liver injury by rFasL in *P. acnes*-primed mice.

To confirm that IL-18 induces liver injury in *P. acnes*-primed mice, we sequentially administered *P. acnes* and IL-18 into wild-type C57BL/6 mice. As shown in Figure 5A, acute liver injury was induced in either wild-type or IL-18-deficient mice, indicating that some effector mechanism for liver injury that acts downstream of IL-18 is intact in IL-18-deficient mice.

To investigate whether endogenous FasL is involved in IL-18-induced liver injury, we administered neutralizing anti-mouse FasL mAb at IL-18 challenge. As shown in Figure 5B, left, anti-murine FasL mAb partially inhibited the liver injury. We also sequentially administered *P. acnes* and IL-18 into C57BL/6 *gld/gld* (*gld/gld*) mice, lacking functional FasL (Takahashi et al., 1994). As shown in Figure 5B, right, the liver injury in *gld/gld* mice was significantly milder than that in wild-type mice. Since IL-18 induces production of IFNγ, we also examined a possible involvement of IFNγ in the *P. acnes*/IL-18-induced liver injury. As shown in Figure 5B, IFNγ-deficient mice showed milder liver injury than wild-type mice. These results suggested that IL-18-induced liver injury is at least partly mediated by both endogenous FasL and IFNγ, the latter of which may then stimulate macrophages to produce a powerfully hepatotoxic factor, TNFα, as shown previously (Tsutsui et al., 1997).

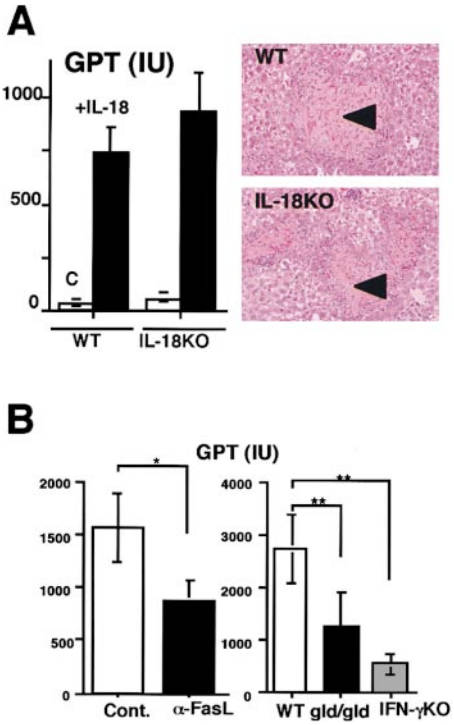

Figure 5. IL-18-Induced Acute Liver Injury

(A) Induction of acute liver injury by IL-18 in *P. acnes*-primed mice. IL-18 (500 ng, closed column) or control PBS (open column) was i.p. administered into *P. acnes*-primed wild-type C57BL/6 mice (WT) or IL-18-deficient mice (IL-18KO). After 24 hr, the serum was sampled for measuring liver enzyme levels (left), and the liver specimens were subjected to HE staining (right). Arrows indicate necrotic lesion. A representative lesion of one mouse among five mice in each experimental group is shown. Similar results were obtained in three independent experiments.

(B) Involvement of endogenous FasL and IFNγ in IL-18-induced liver injury. Left, *P. acnes*-primed wild-type C57BL/6 mice were i.p. administered with IL-18 (500 ng) in the presence of neutralizing anti-mouse FasL mAb (α-FasL, closed column) or control IgG (Cont., open column). Serum GPT levels were determined 24 hr after. Data are indicated as mean ± SD of five mice in each group and representative of three independent experiments with similar results. Asterisk, $p < 0.05$. Right, *P. acnes*-primed wild-type C57BL/6 mice (WT, $n = 20$), *gld/gld* mice (gld/gld, $n = 20$), or IFNγ-deficient mice (IFNγKO, $n = 11$) were i.p. administered with IL-18 (500 ng). Serum GPT levels were determined 24 hr after. Data are indicated as mean ± SD. Double asterisks, $p < 0.01$.

Discussion

In the present study, we demonstrated that *P. acnes*-primed macrophages secrete biologically active IL-18 upon stimulation with FasL. The *P. acnes* priming rendered macrophages responsive to FasL stimulation by upregulating Fas expression in an IFNγ-dependent manner. In contrast to the LPS-stimulated IL-18 secretion that was strictly caspase-1 dependent, the FasL-stimulated IL-18 secretion appeared to be mediated by some caspase other than caspase-1. In addition, we revealed that the FasL-stimulated IL-18 secretion from primed macrophages plays a critical role in the pathogenesis of FasL-induced liver injury.

It has been shown that IL-18 secretion from LPS-stimulated macrophages requires processing by caspase-1 (Ghayur et al., 1997; Gu et al., 1997). We here also observed that *P. acnes*-primed macrophages from caspase-1-deficient mice did not secrete IL-18 upon LPS stimulation (Figure 1). In contrast to the caspase-1-dependent IL-18 secretion upon LPS stimulation, the FasL-stimulated IL-18 secretion was totally caspase-1 independent (Figure 1). A similar caspase-independent processing of IL-1β has also been noted in FasL-stimulated neutrophils (Miwa et al., 1998). In both cases, processing was blocked by caspase inhibitors, and secretion of these cytokines did not correlate to apoptosis (Miwa et al., 1998; Figure 2B). To date, the only caspases known to cleave pro-IL-18 are caspase-1, caspase-4, and caspase-3, while caspase-3 leads to production of biologically inactive fragments (Akita et al., 1997; Gu et al., 1997). Biochemical characterization of IL-18 secreted from FasL-stimulated, caspase-1-deficient macrophages showed almost the same size as the conventional IL-18 secreted from LPS-stimulated macrophages (Figure 2A), suggesting that alternative processing enzyme might have caspase-1-like specificity. It has been reported that cotransfection of caspase-4 and proIL-18 into COS cells resulted in intracellular cleavage of proIL-18 into biologically active IL-18 of 18 kDa (Gu et al.,

1997). Therefore, caspase-4 seems to be the most likely candidate. Interestingly, proIL-1β also can be cleaved by multiple enzymes including caspase-1, and the cleavage sites cluster around the site cleaved by caspase-1 (Fantuzzi et al., 1997). Alternatively, a protease(s) activated by caspases would process IL-18 in FasL-stimulated macrophages. Recently, proteinase-3, which is a serine protease stored in cytoplasmic granules of neutrophils and monocytes, has been reported to be an alternative processing enzyme for IL-1β and IL-18 (Coeshott et al., 1999; Fantuzzi and Dinarello, 1999). Further studies are now under way to characterize the molecular mechanism for caspase-1-independent IL-18 secretion from FasL-stimulated macrophages.

It has been reported that liver injury could not be readily induced by the administration of rFasL unless the mice had been primed with *P. acnes* (Tanaka et al., 1997). However, the reasons why only *P. acnes*-primed mice are sensitive to FasL-induced liver injury have not been completely understood. Here, we demonstrated that one of the roles of *P. acnes* treatment is to induce functional Fas expression on macrophages, presumably by endogenous accumulation of IFNγ. As previously reported (Matsui et al., 1997), *P. acnes*-elicited Kupffer cells secrete IL-12, which stimulates local NK cells and T cells to produce IFNγ that in turn stimulates Kupffer cells to upregulate their functional Fas expression (Figure 3). In fact, Fas expression was not observed in macrophages derived from *P. acnes*-primed IFNγ-deficient mice (Figure 3). Consequently, IL-18 serum level did not increase in these mice lacking Fas-expressing macrophages even after administration of rFasL (Figure 4A). In addition, *P. acnes* treatment induces multiple granuloma formation mainly consisting of macrophages/Kupffer cells and lymphocytes in the liver as recently shown (Sakao et al., 1999). Indeed, the number of Kupffer cells and splenic macrophages of *P. acnes*-treated mice was elevated by about 20- and 5-fold, respectively, in those from nontreated mice (data not shown). This accumulation of primed Kupffer cells and macrophages in the liver may also contribute to local secretion of a large amount of IL-18 upon FasL stimulation, which leads to acute liver injury.

Our present study suggests a critical role of IL-18 in FasL-induced liver injury, as represented by the inability of rFasL to cause liver injury in *P. acnes*-primed IL-18-deficient mice (Figure 4B). FasL has been implicated in liver injury associated with various animal models of hepatitis and liver diseases, such as viral hepatitis, alcoholic liver cirrhosis, and Wilson's disease (Hayashi and Miwa, 1997; Kondo et al., 1997; Seino et al., 1997; Tagawa et al., 1998; Taeb et al., 1998; Strand et al., 1998). Since FasL is directly cytotoxic to hepatocytes in vitro, it has been generally supposed that the predominant mode of FasL action is direct cytotoxicity against liver parenchymal cells (Seino et al., 1997; Zheng et al., 1998). However, our present results showed that soluble FasL did not exert direct hepatocytotoxicity in IL-18-deficient mice even after *P. acnes* priming. Although soluble FasL can be efficiently secreted from activated T cells (Kayagaki et al., 1995), it has been shown that cytotoxic activity of soluble FasL is greatly reduced as compared to membrane-bound FasL (Oyaizu et al., 1997; Tanaka et al., 1998). Therefore, the predominant mode of soluble FasL action appears to induce IL-18 secretion from macrophages rather than to directly induce hepatocyte apoptosis. We have shown that IL-18 augments cytotoxic activities of NK cells and T cells by upregulating membrane FasL expression on these cells and by enhancing perforin-mediated cytotoxicity (Dao et al., 1996; Tsutsui et al., 1996; Hyodo et al., 1999). Our present results demonstrate that IL-18-induced liver injury was at least partly mediated by endogenous FasL and IFNγ possibly induced by IL-18 (Figure 5B). Therefore, the soluble FasL-stimulated IL-18 secretion from macrophages plays an amplifying role in the development of liver injury.

Administration of IL-18 solely induced liver injury in *P. acnes*-primed mice (Figure 5A), whereas it did not cause any liver damage in nontreated mice (data not shown). This is partly because *P. acnes* treatment induces responsiveness of hepatic lymphocytes to the stimulation with IL-18 (Matsui et al., 1997). As previously reported, splenic T cells require IL-12 stimulation to express IL-18R (Yoshimoto et al., 1998). This is also the case for hepatic lymphocytes. Hepatic T cells from nontreated mice do not express IL-18R. However, *P. acnes*-primed hepatic T cells expressed IL-18R on their surface, as determined by flow cytometry (K. M., unpublished data). IL-18-induced liver injury is partly due to the upregulation of endogenous FasL expression and also to the induction of IFNγ production, presumably leading to the sequential activation of proinflammatory cytokine cascade, as previously reported (Tsutsui et al., 1997) (Figure 5B). Additionally, IFNγ plays a critical role in upregulation of Fas on Kupffer cells. Therefore, IFNγ contribute to liver injury by induction of Fas at priming phase and of TNFα production at effector phase.

Here, we demonstrate that the activation of Fas induces IL-18 secretion from IFNγ-primed macrophages. IFNγ plays a critical role in host defense against infection with intracellular microbes (Cooper et al., 1993; Dai et al., 1997). IL-18 and IL-12 participate in host defense as potent upstream cytokines to IFNγ. IL-18 conferred resistance to *Salmonella typhimurium* and *Cryptococcus neoformans* (Kawakami et al., 1997; Zhang et al., 1997; Mastroeni et al., 1999). In such infection, IL-18 may upregulate functional FasL expression on NK cells and Th1 cells (Dao et al., 1996; Tsutsui et al., 1996) and also induce production of IFNγ by these cells, which in turn upregulates functional Fas expression on macrophages, leading to amplification of IL-18 production. This positive circuit between IL-18 and Fas/FasL system may cause rapid and massive production of IFNγ, leading to effective clearance of microbes. Functional Fas or FasL mutant mice were reported to be sensitive to *Leishmania major* infection (Conceiçao-Silva et al., 1998). This may be explained by abrogation of FasL-mediated IL-18 secretion in Fas- or FasL-disrupted mice. Thus, the FasL-stimulated IL-18 secretion from IFNγ-primed macrophages may play an important role in amplifying the Th1-mediated inflammatory responses. However, an excessive acceleration of IL-18 production by this circuit may also cause inflammatory diseases such as hepatitis and rheumatoid arthritis. We are now investigating pathophysiological roles of the Fas/FasL-mediated IL-18 secretion in various inflammatory diseases.

Experimental Procedures

Mice and Reagents

Female C57BL/6 mice (6- to 8-week-old) and female C57BL/6 *gld/gld* mice (6- to 8-week-old) were purchased from Japan SLC (Shizuoka, Japan). Caspase-1-deficient mice (Kuida et al., 1995) were backcrossed with C57BL/6 mice, and F5 (5- to 8-week-old, female) were used for this study. IL-18-deficient mice (Takeda et al., 1998) were also backcrossed with C57BL/6 mice, and F8 (5- to 8-week-old, female) were used. IFNγ-deficient mice (5- to 8-week-old, female) on C57BL/6 background were kindly provided by Dr. Y. Iwakura of Tokyo University (Tokyo, Japan) (Tagawa et al., 1997; Hyodo et al., 1999). Heat-killed *P. acnes* was prepared as previously described (Tsutsui et al., 1997). The mice that had been i.p. administered with 1 mg of *P. acnes* 7 days before were used as *P. acnes*-primed mice. Neutralizing mAbs against murine FasL (MFL-1, hamster IgG, and K10, mouse IgG2b) and human FasL (NOK-1, mouse IgG1) and murine mFasL cells were generated as previously described (Kayagaki et al., 1995, 1997). rFasL was prepared as previously reported (Oyaizu et al., 1997). LPS derived from *Escherichia coli* 055:B5 was purchased from Difco. ZVAD and YVAD were purchased from the Peptide Institute (Osaka, Japan). Recombinant IL-2 was kindly provided by Shionogi (Osaka, Japan). Normal hamster IgG and control mouse IgG1 were purchased from PharMingen. Constitutively FasL-expressing hepatic NK cell line, LNK5E3, was established from *P. acnes*-primed BALB/c *nu/nu* mice, as previously reported (Tsutsui et al., 1996). Culture medium generally used in this study was RPMI-1640 supplemented with 10% FCS, 100 U/ml of penicillin, 100 μg/ml of streptomycin, 50 μM 2-ME, and 2 mM L-glutamine.

Preparation of Macrophages

Spleen cells (5×10^6/ml) isolated from nontreated or *P. acnes*-primed mice were incubated in 10 cm dishes for 1 hr, and adherent cells were harvested for splenic macrophages. Kupffer cells were also isolated from the variously treated mice, as shown previously (Tsutsui et al., 1997). The macrophages (1×10^6/ml) were incubated with 1 μg/ml of LPS, 2×10^6/ml of mFasL cells, 2×10^6/ml of LNK5E3 cells, or various concentrations of rFasL in the presence or absence of 10 μg/ml of anti-mouse FasL mAb or 50 μg/ml of anti-human FasL mAb for 24 hr. The macrophages were also incubated with various doses of rFasL in the presence or absence of 20 μM ZVAD or YVAD for 24 hr. In some experiments, macrophages were incubated with 20 ng/ml of rFasL for 24 hr, and the cells were collected for analysis of apoptosis by flow cytometry. The culture supernatants were assayed for IL-18 concentration by ELISA and IL-18 activity by a bioassay, as described below.

Metabolic Labeling and Immunoprecipitation

Metabolic labeling for IL-18 was performed as previously reported (Gu et al., 1997). In brief, Kupffer cells from *P. acnes*-primed C57BL/6 mice or caspase-1-deficient mice were starved by preculture in methionine/cysteine-free culture medium (Dainippon Pharmaceutical, Tokyo, Japan) and then incubated with [^{35}S]Cys/Met (TransLabel; ICN Biomedical) in the presence of 1 μg/ml of LPS or 20 ng/ml of rFasL for 5 hr, and supernatant was collected. After preclearing with normal rabbit Ig-coupled Sepharose (Pharmacia, Uppsala, Sweden), resulting supernatant was immunopretipitated with anti-IL-18 Ab-coupled Sepharose. The materials bound to Sepharose were analyzed by SDS-PAGE under reducing conditions.

Flow Cytometric Analysis

Fas expression on splenic macrophages or Kupffer cells from variously treated mice were determined by FACS. The cells were incubated with PE-conjugated anti-Fas mAb (PharMingen) and FITC-conjugated anti-Mac1 mAb (CD11b) (PharMingen) following FcR blocking using anti-FcgR mAb (PharMingen). Stained cells were analyzed using a dual laser FACScalibur (Becton Dickinson). Ten thousand cells were analyzed, and data were processed with CellQuest (Becton Dickinson).

Biological Assay for IL-18

To determine the biological activity of IL-18, IL-18-responsive cloned hepatic NK cells, LNK5E3, were used (Tsutsui et al., 1997). IL-18 and IL-12 synergistically induce IFNγ production by LNK5E3 cells (data not shown). To increase sensitivity of the assay, we measured IL-18 activity in the presence of a small amount of IL-12 that alone did not induce IFNγ production by LNK5E3. LNK5E3 cells (5×10^5/ml) were incubated with 100 pg/ml of IL-12, 500 U/ml of IL-2, and 25% macrophage supernatants, or 10% serum from variously treated mice in the presence or absence of 100 μg/ml anti-IL-18 antibody, which completely inhibited 100 ng/ml of recombinant murine IL-18, for 48 hr. IFNγ in the culture supernatant was determined by ELISA (Genzyme). IL-18 activity was defined as followed: IL-18 activity (U/ml) = (IFNγ [pg/ml] in LNK5E3 supernatant without anti-IL-18 antibody) - (IFNγ [pg/ml] in the supernatant with anti-IL-18 antibody).

ELISA for Cytokines

IL-18 was measured by ELISA kits kindly provided by Hayashibara (Okayama, Japan), as previously reported (Matsui et al., 1997).

Assay for Apoptosis

The ratio of apoptotic cells in variously treated macrophages was determined by nuclear staining with propiodium iodide followed by FACS analysis as previously reported (Nakanishi et al., 1996).

Induction of Liver Injury

rFasL (500 ng) in PBS was i.v. administered into *P. acnes*-primed or nontreated mice with various genetic backgrounds. In some experiments, *P. acnes*-primed C57BL/6 mice were i.p. administered with 1 mg of anti-human FasL mAb 1 hr before the administration of rFasL. For one experimental group, five mice were used. At 24 hr, the serum was collected for determining IL-18 and liver enzyme levels, and the liver specimens were sampled for histological study. The liver specimens were fixed and stained with HE. In some experiments, IL-18 (500 ng) was i.p. injected into wild-type C57BL/6 mice along with 1 mg of anti-mFasL mAb (K10) or control mouse IgG at day 5, 6, and 7 after the administration of *P. acnes*. For one experimental group, five mice were used. At 24 hr, sera were sampled for determination of liver enzyme levels. In some experiments, IL-18 (500 ng) was i.p. injected into wild-type C57BL/6 mice ($n = 20$), *gld/gld* mice ($n = 20$), or IFNγ-deficient mice ($n = 11$) at day 5, 6, and 7 after the administration of *P. acnes*. At 24 hr, the sera were sampled for determination of liver enzyme levels.

Statistics

All data are given as mean ± SD. Significance between the control group and a treated group was examined with the unpaired Student's t test. P values less than 0.05 were regarded as significant.

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References

Akita, K., Ohtsuki, T., Nukada, Y., Tanimoto, T., Namba, M., Okura, T., Takayama-Yamamoto, R., Torigoe, K., Gu, Y., Su, M.S.S., et al. (1997). Involvement of caspase-1 and caspase-3 in the production and processing of mature human interleukin 18 in monocytic THP.1 cells. J. Biol. Chem. *272*, 26595–26603.

Bazan, J.F., Timans, J.C, and Kastelein, R.A. (1996). A newly defined interleukin-1? Nature *379*, 591.

Born, T.L., Thomasson, E., Bird, T.A., and Sims, J.E. (1998). Cloning of a novel receptor subunit, AcPL, required for interleukin-18 signaling. J. Biol. Chem. *273*, 29445–29450.

Coeshott, C., Ohnemus, C., Pilyavskaya, A., Ross, S., Wieczorek, M., Kroona, H., Leimer, A.H., and Cheronis, J. (1999). Converting enzyme-independent release of tumor necrosis factor α and IL-1β from a stimulated human monocytic cell line in the presence of activated neutrophils or purified proteinase 3. Proc. Natl. Acad. Sci. USA. *96*, 6261–6266.

Conceiçao-Silva, F., Hahne, M., Schröter, M., Louis, J., and Tschopp, J. (1998). The resolution of lesions induced by *Leishmania major* in mice requires a functional Fas (APO-1, CD95) pathway of cytotoxicity. Eur. J. Immunol. *28*, 237–245.

Cooper, A.M., Dalton, D.K., Stewart, T.A., Griffin, J.P., Russell, D.G., and Orme, I.M. (1993). Disseminated tuberculosis in interferon γ gene-disrupted mice. J. Exp. Med. *178*, 2243–2247.

Dai, W.J., Garnens, W., Kohler, G., Hufnagel, M., Kopf, M., and Brombacher, F. (1997). Impaired macrophage listericidal and cytokine activities are responsible for the rapid death of Listeria monocytogenes-infected IFN-γ receptor-deficient mice. J. Immunol. *158*, 5297–5304.

Dao, T., Ohashi, K., Kayano, T., Kurimoto, M., and Okamura, H. (1996). Interferon-gamma-inducing factor, a novel cytokine, enhances Fas ligand-mediated cytotoxicity of murine T helper 1 cells. Cell. Immunol. *173*, 230–235.

Dinarello, C.A., Novick, D., Puren, A.J., Fantuzzi, G., Shapiro, L., Mühl, H., Yoon, D., Reznikov, L.L., Kim, S., and Rubinstein, M. (1998). Overview of interleukin-18: more than an interferon-γ inducing factor. J. Leuk. Biol. *63*, 658–664.

Fantuzzi, G., and Dinarello, C.A. (1999). Interleukin-18 and interleukin-1β: two cytokine substrates for ICE (caspase-1). J. Clin. Immunol. *19*, 1–11.

Fantuzzi, G., Ku, G., Harding, M.W., Livingston, D.J., Sipe, J.D., Kuida, K., Flavell., R.A., and Dinarello, C.A. (1997). Response to local Inflammation of IL-1β-converting enzyme-deficient mice. J. Immunol. *158*, 1818–1824.

Ghayur, T., Banerjee, S., Hugunin, M., Butler, D., Herzog, L., Carter, A., Quinta, L., Sekut, L., Talanian, R., Paskind, M., et al. (1997). Caspase-1 processed IFN-γ-inducing factor and regulates LPS-induced IFN-γ production. Nature *386*, 619–623.

Gu, Y., Kuida, K., Tsutsui, H., Ku, G., Hsiao, K., Fleming, M.A., Hayashi, N., Higashino, K., Okamura, H., Nakanishi, K., et al. (1997). Activation of interferon-g inducing factor mediated by interleukin-1b converting enzyme. Science *275*, 206–1434.

Hayashi, N., and Miwa, E. (1997). Fas system and apoptosis in viral hepatitis. J. Gastroenterol. Hepatol. *12*, S223–S226.

Hyodo, Y., Matsui, K., Hayashi, N., Tsutsui, H., Kashiwamura, S-I., Yamauchi, Y., Hiroishi, K., Takeda, K., Tagawa, Y., Iwakura, Y., et al. (1999). IL-18 up-regulates perforin-mediated NK activity without increasing perforin messenger RNA expression by binding to constitutively expressed IL-18 receptor. J. Immunol. *162*, 1662–1668.

Kawakami, K., Qureshi, M.H., Zhang, T., Okamura, H., Kurimoto, H., and Saito, A. (1997). IL-18 protects mice against pulmonary and disseminated infection with *Cryptococcus neoformans* by inducing IFN-γ production. J. Immunol. *159*, 5528–5534.

Kayagaki, N., Kawasaki, A., Ebata, T., Ohmoto, H., Ikeda, S., Inoue, S., Yoshino, K., Okumura, K., and Yagita, H. (1995). Metalloproteinase-mediated release of human Fas ligand. J. Exp. Med. *182*, 1777–1783.

Kayagaki, N., Yamaguchi, N., Nagao, F., Matsuo, S., Maeda, H., Okumura, K., and Yagita, H. (1997). Polymorphism of murine Fas ligand that affects the biological activity. Proc. Natl. Acad. Sci. USA *94*, 3914–3919.

Kondo, T., Suda, T., Fukuyama, H., Adachi, M., and Nagata, S. (1997). Essential roles of the Fas ligand in the development of hepatitis. Nat. Med. *4*, 409–413.

Kuida, K., Lippke, J.A., Ku, G., Harding, M.W., Livingston, D.J., Su, M.S.-S., and Flavell, R.A. (1995). Altered cytokine expert and apoptosis in mice deficient in interleukin-1β converting enzyme. Science *267*, 2000–2003.

Mastroeni, P., Clare, S., Khan, S., Harrison, J.A., Hormaeche, C.E., Okamura, H., Kurimoto, M., and Dougan. G. (1999). Interleukin 18 contributes to host resistance and gamma interferon production in mice infected with virulent *Salmonella typhimurium*. Infect. Immun. *67*, 478–483.

Matsui, K., Yoshimoto, T., Tsutsui, H., Hyodo, Y., Hayashi, N., Hiroishi, K., Kawada, N., Okamura, H., Nakanishi, K., and Higashino, K. (1997). Propionibacterium acnes treatment diminished $CD4^+NK1.1^+$ T cells but induces type 1 T cells in the liver by induction of IL-12 and IL-18 production from Kupffer cells. J. Immunol. *159*, 97–106.

Miwa, K., Asano, M., Horai, R., Iwakura, Y., Nagata, S., and Suda, T. (1998). Caspase-1-independent IL-1β release and inflammation induced by the apoptosis inducer Fas ligand. Nat. Med. *4*, 1287–1292.

Nakanishi, K., Matsui, K., Kashiwamura, S.-I., Nishioka, Y., Nomura, J., Nishimura, Y., Sakaguchi, N., Yonehara, S., Higashino, K., and Shinka, S. (1996). IL-4 and anti-CD40 protect against Fas-mediated B cell apoptosis and induce B cell growth and differentiation. Int. Immunol. *8*, 791–798.

Okamura, H., Tsutsui, H., Komatsu, T., Yutsudo, M., Hakura, A., Tanimoto, T., Torigoe, K., Okura, T., Nukada, Y., Hattori, K., et al. (1995). Cloning of a new cytokine that induces IFN-γ production by T cells. Nature *378*, 88–91.

Okamura, H., Tsutsui, H., Kashiwamura, S., Yoshimoto, T., and Nakanishi, K. (1998a). Interleukin-18 (IL-18): a novel cytokine that augments both innate and acquired immunity. Adv. Immunol. *70*, 281–312.

Okamura, H., Kashiwamura, S.-I., Tsutsui, H., Yoshimoto, T., and Nakanishi, K. (1998b). Regulation of interferon-γ production by IL-12 and IL-18. Curr. Opin. Immunol. *10*, 259–264.

Oyaizu, N., Kayagaki, N., Yagita, H., Pahwa, S., and Ikawa, Y. (1997). Requirement of cell-cell contact in the induction of Jurkat T cell apoptosis: the membrane-anchored but not soluble form of FasL can trigger anti-CD3-induced apoptosis in Jurkat T cells. Biochem. Biophys. Res. Commun. *238*, 670–675.

Sakao, Y., Takeda, K., Tsutsui, H., Kaisho, T., Nomura, F., Okamura, H., Nakanishi, K., and Akira, S. (1999). IL-18-deficient mice are resistant to endotoxin-induced liver injury but highly susceptible to endotoxin shock. Int. Immunol. *11*, 471–480.

Seino, K., Kayagaki, N., Takeda, K., Fukao, K., Okumura, K., and Yagita, H. (1997). Contribution of Fas ligand to T cell-mediated hepatic injury in mice. Gastroenterology *113*, 1315–1322.

Spanaus, K.S., Schlapbach, R., and Fontana, A. (1998). TNF-alpha and IFN-gamma render microglia sensitive to Fas ligand-induced apoptosis by induction of Fas expression and down-regulation of Bcl-2 and Bcl-xL. Eur. J. Immunol. *28*, 4398–4408.

Strand, S., Hofmann, W.J., Grambihler, A., Hug, H., Volkmann, M., Otto, G., Wesch, H., Mariani, S.M., Hack, V., Stremmel, W., et al. (1998). Hepatic failure and liver cell damage in acute Wilson's disease involve CD95 (APO-1/Fas) mediated apoptosis. Nat. Med. *4*, 588–593.

Tagawa, Y., Sekikawa, K., and Iwakura, Y. (1997). Suppression of Concanavalin A-induced hepatitis in IFN-$\gamma^{-/-}$ mice, but not in TNF-$\alpha^{-/-}$ mice. J. Immunol. *159*, 1418–1428.

Tagawa, Y., Kakuta, S., and Iwakura, Y. (1998). Involvement of Fas/Fas ligand system-mediated apoptosis in the development of concanavalin A-induced hepatitis. Eur. J. Immunol. *28*, 4105–4113.

Taeb, J., Mathurin, P., Poynard, T., Gougerot-Pocidalo, M.A., and Chollet-Martin, S. (1998). Raised plasma soluble Fas and Fas ligand in alcoholic liver disease. Lancet *351*, 1930–1931.

Takahashi, T., Tanaka, M., Brannan, C.I., Jenkins, N.A., Copeland, N.G., Suda, T., and Nagata, S. (1994). Generalized lymphoproliferative disease in mice, caused by a point mutation in the Fas ligand. Cell *76*, 969–976.

Takeda, K., Tsutsui, H., Yoshimoto, T., Adachi, O., Yoshida, N., Kishimoto, T., Okamura, H., Nakanishi, K., and Akira, S. (1998). Defective NK cell activity and Th1 response in IL-18-deficient mice. Immunity *8*, 383–390.

Tanaka, M., Suda, T., Yatomi, T., Nakamura, N., and Nagata, S. (1997). Lethal effect of recombinant human Fas ligand in mice pretreated with Propionibacterium acnes. J. Immunol. *158*, 2303–2309.

Tanaka, M., Itai, T., Adach, M., and Nagata, S. (1998). Downregulation of Fas ligand by shedding. Nat. Med. *4*, 31–36.

Torigoe, K., Ushio, S., Okura, T., Kobayashi, S., Taniai, M., Kunikata, T., Murakami, T., Sanou, O., Kojima, H., Fujii, M., et al. (1997). Purification and characterization of the human Interleukin-18 (hIL-18) receptor. J. Biol. Chem. *272*, 25737–25742.

Tsutsui, H., Mizoguchi, Y., and Morisawa, S. (1992). Importance of direct hepatocytolysis by liver macrophages in experimental fulminant hepatitis. Hepato-Gastroenterology *39*, 553–559.

Tsutsui, H., Nakanishi, K., Matsui, K., Higashino, K., Okamura, H., Miyazawa, Y., and Kaneda, K. (1996). IFN-γ-inducing factor up-regulates Fas ligand-mediated cytotoxic activity of murine natural killer cell clones. J. Immunol. *157*, 3967–3973.

Tsutsui, H., Matsui, K., Kawada, N., Hyodo, Y., Hayashi, N., Okamura, H., Higashino, K., and Nakanishi, K. (1997). IL-18 accounts for both TNF-α- and Fas ligand-mediated hepatotoxic pathways in endotoxin-induced liver injury in mice. J. Immunol. *159*, 3961–3967.

Yoshimoto, T., Takeda, K., Tanaka, T., Ohkusu, K., Kashiwamura, S., Okamura, H., Akira, S., and Nakanishi, K. (1998). IL-12 up-regulates IL-18R expression on T cells, Th1 cells and B cells: synergism with IL-18 for IFN-γ production. J. Immunol. *161*, 3400–3407.

Zhang, T., Kawakami, K., Qureshi, M.H., Okamura, H., Kurimoto, M., and Saito, A. (1997). Interleukin-12 (IL-12) and IL-18 synergistically induce the fungicidal activity of murine peritoneal exudate cells against *Cryptococcus neoformans* through production of gamma interferon by natural killer cells. Infect. Immun. *65*, 3594–3599.

Zheng, T.S., Schlosser, S.F., Dao, T., Hingorani, R., Crispe, I.N., Boyer, J.L., and Flavell, R.A. (1998). Caspase-3 controls both cytoplasmic and nuclear events associated with Fas-mediated apoptosis in vivo. Proc. Natl. Acad. Sci. USA *95*, 13618–13623.