Fas ligand–induced caspase-1–dependent accumulation of interleukin-18 in mice with acute graft-versus-host disease

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Acute graft-versus-host disease (aGVHD), the fatal side effects of bone marrow transplantation, was shown to be accompanied by elevation of serum levels of interleukin-18 (IL-18). In this study, the mechanism underlying the accumulation of IL-18 in aGVHD in mice was investigated. Lethally irradiated recipients having transplantation with H-2 disparate donor spleen cells demonstrated aGVHD and contained markedly elevated serum levels of IL-18. In contrast, recipients having transplantation with gld/gld spleen cells, which lack functional Fas ligand (FasL), contained only normal ranges of IL-18, indicating FasL-mediated IL-18 release in aGVHD. The wild-type hosts engrafted with caspase-1–deficient cells revealed marked increases of IL-18 similar to those engrafted with wild-type cells, whereas caspase-1–deficient recipients engrafted with wild-type cells showed only a slight elevation of serum IL-18, indicating that IL-18 elevation is derived from host cells in a caspase-1–dependent manner. These results suggest FasL-mediated caspase-1–dependent IL-18 secretion in aGVHD in mice.

Introduction

Interleukin 18 (IL-18) is a cytokine with wide-ranging biologic functions that include activation not only of innate immunity, but also of acquired immunity, including both Th1 responses, particularly in collaboration with IL-12, and Th2 responses. Furthermore, IL-18 is involved in the development of cytotoxic T lymphocytes and natural killer cells. The regulatory mechanism of IL-18 secretion is distinct from that of usual secretory cytokines. IL-18 is stored as biologically inactive precursor (pro–IL-18) and is secreted after cleavage by appropriate cutting enzymes. Caspase-1 is a prerequisite for the secretion of IL-18 upon activation by certain stimuli, including lipopolysaccharide (LPS). Caspase-1–like is required for the secretion of IL-18 from Propionibacterium acnes–elicited Kupffer cells after stimulation with Fas ligand (FasL), although this study does not exclude the possible involvement of caspase-1. Thus, the precise regulatory mechanism of IL-18 secretion is still uncertain. Recently, we and others have shown that the serum concentration of IL-18 is elevated in patients with acute graft-versus-host disease (aGVHD). In aGVHD, the Fas/FasL system plays an essential role in the development of fatal tissue injuries. Here, we investigated the mechanism underlying the accumulation of IL-18 in a mouse model of aGVHD and found that IL-18 is secreted in a FasL-initiated, caspase-1–dependent manner.

Study design

Mice

C57BL/6 (B6) mice, B6XD2A2/F1 (BDF1) mice, and BALB/c mice were purchased from SLC (Shizuoka, Japan). B6 gld/gld mice were purchased from CLEA Japan (Osaka, Japan). Caspase-1–deficient mice and wild-type (WT) littermates in a mixture of 129 and B6 background (B6/129) were used. Caspase-1–deficient mice were back-crossed with BALB/c mice, and F8 mice were used. All of the mice used (females, 6-10 weeks old) were kept under specific pathogen-free conditions.

Reagents

L5178Y cells transfected with mouse FasL (mFasL) and neutralizing anti-mouse FasL monoclonal antibody (mAb; MFL-1, hamster IgG) were kindly provided by Dr N. Kayagaki (Juntendo University, Tokyo, Japan). LPS derived from Escherichia coli 055:B5 was purchased from Difco (Detroit, MI). Culture medium generally used in this study was RPMI-1640 (Wako Pure Chemical, Osaka, Japan). Caspase-1–deficient mice and wild-type (WT) littermates in a mixture of 129 and B6 background (B6/129) were used. Caspase-1–deficient mice were back-crossed with BALB/c mice, and F8 mice were used. All of the mice used (females, 6-10 weeks old) were kept under specific pathogen-free conditions.

Induction of aGVHD

aGVHD was induced by intravenous injection of 5 × 10^7 viable, unfractionated donor spleen cells in lethally irradiated (9 Gy) recipients. Control mice received syngeneic (syn) spleen cells (5 × 10^7).

Assay for IL-18

The concentration of IL-18 was determined by an enzyme-linked immunosorbent assay (ELISA) kit (MBL, Nagoya, Japan).

Preparation of Kupffer cells

Kupffer cells were isolated from variously treated mice as shown previously. Kupffer cells (1 × 10^6/mL) were incubated with mFasL.
(1 × 10⁶/mL) in the presence of 20 μg/mL anti-mouse FasL or with 1 μg/mL LPS for 24 hours.

**Statistical analysis**

The Student t test or the Fisher protected least significant difference test was performed. P < .05 was considered statistically significant.

**Results and discussion**

**Elevated serum levels of IL-18 in aGVHD mice**

As reported previously, levels of serum IL-18 are elevated in patients with aGVHD. This was also the case for mouse aGVHD. As shown in Figure 1A, IL-18 serum levels increased after transplantation of WT B6 spleen cells in 9 Gy-irradiated BDF1 mice (aGVHD-induced mice) and reached a plateau at day 10 (P < .0001 versus syngeneic controls). The serum levels of IL-18 correlated with the donor cell number infused, and the duration required for reaching their peaks became shortened (data not shown). Furthermore, there is a correlation between the concentration of serum IL-18 and the severity of pathologic changes in aGVHD target organs, such as spleen, liver, and intestines (H. I., unpublished data, August 2000). In contrast, IL-1β, which is processed by the same enzymes that cleave pro-IL-18, was not elevated in the serum of aGVHD-induced mice (data not shown). Therefore, high serum levels of IL-18 but not IL-1β seem to be an indicator of aGVHD not only in human patients, but also in mouse experimental models.

**Fas/FasL-mediated IL-18 secretion in aGVHD**

As reported previously, Fas-expressing macrophages release biologically active IL-18 upon stimulation with FasL, depending on a non-caspase-1, caspase-dependent manner. It has also been demonstrated that FasL-expressing cells were induced after induction of aGVHD. To investigate whether the Fas/FasL system is involved in the secretion of IL-18 in aGVHD, we transplanted splenocytes from gld/gld B6 mice, which lack functional FasL. As shown in Figure 1B, BDF1 mice having transplantation with gld/gld splenocytes did not show elevated serum levels of IL-18 compared with those engrafted with WT splenocytes (P < .01). No obvious increase in serum levels of IL-18 was observed by day 21 after transplantation with gld/gld splenocytes (data not shown). Furthermore, when 10-fold splenocytes from gld/gld B6 mice were transplanted into BDF1 mice, no significant elevation of serum IL-18 levels was observed by day 14 (data not shown). These results strongly suggest that IL-18 in aGVHD accumulates in a Fas/FasL-mediated fashion.

Next, we investigated what cell types secrete IL-18 upon stimulation with FasL. To address this, we prepared Kupffer cells (tissue macrophages in the liver) from aGVHD mice and incubated them with FasL-expressing cells in vitro. As shown in Figure 1C, Kupffer cells from aGVHD mice secreted IL-18 in response to membrane-associated FasL (column D), which was completely inhibited by neutralizing anti-FasL antibody (column F), indicating that the Kupffer cells secreted IL-18 upon stimulation with FasL. Kupffer cells acquired Fas expression after the induction of aGVHD (data not shown). Thus, the Fas/FasL system seems to play a role not only as an effector molecule involved in various tissue injuries including aGVHD, but also as a regulating factor prerequisite for the secretion of IL-18 in aGVHD.

**Caspase-1-dependent IL-18 secretion after stimulation with FasL**

Although, as we demonstrated previously, caspase-1-deficient Kupffer cells derived from P. acnes–primed mice can secrete IL-18 after stimulation with FasL, it is still possible that FasL stimulation might also activate caspase-1. Therefore, we investigated whether FasL-induced IL-18 accumulation in aGVHD is dependent on caspase-1. To test this possibility, we transplanted caspase-1-deficient or WT B6/129 spleen cells into caspase-1-deficient or WT BALB/c mice, respectively (Figure 2). Caspase-1-deficient hosts having transplantation with caspase-1-deficient cells showed only a slight elevation in serum levels of IL-18 (Figure 2, column D), whereas WT hosts having transplantation with WT cells contained high serum levels of IL-18 (column A), indicating caspase-1-dependent IL-18 release. Furthermore, IL-18 serum levels were also elevated in the recipients engrafted with WT cells, reaching their peaks before those engrafted with caspase-1-deficient cells. These results strongly support the idea that in aGVHD, IL-18 accumulates in a caspase-1-dependent manner, which is processed by the same enzymes that cleave pro–IL-18, 11,18,19 was not elevated in the serum of aGVHD-induced mice (data not shown). Therefore, high serum levels of IL-18 but not IL-1β seem to be an indicator of aGVHD not only in human patients, but also in mouse experimental models.

**Figure 1. FasL-dependent elevation of serum IL-18 levels after induction of aGVHD.** (A) Elevation of IL-18 serum levels after induction of aGVHD. BDF1 mice were lethally irradiated and underwent transplantation with 5 × 10⁶ spleen cells from BDF1 (Syn), or WT B6 (aGVHD), or WT B6 mice. At the indicated day, the serum was sampled for measurement of IL-18 concentration by ELISA. Data represent the mean ± SD of 5 mice in each experimental group. *P < .0001 by Fisher PLSD test. Similar results were obtained in 3 independent experiments. (B) Lack of increase in serum levels of IL-18 in the recipients engrafted with gld/gld spleen cells. Lethally irradiated BDF1 mice had transplantation with 5 × 10⁶ spleen cells from BDF1 mice (Syn), WT B6 mice (aGVHD), or gld/gld B6 mice (gld/gld). At day 10, serum levels of IL-18 were measured by ELISA. Data represent the mean ± SD of 5 mice in each experimental group. Similar results were obtained in 3 independent experiments. (C) Fas/FasL-dependent IL-18 secretion in vitro by Kupffer cells from aGVHD hosts. Kupffer cells were prepared from BDF1 mice having transplantation with BDF1 spleen cells (Syn; A,C,E,G) or WT B6 cells (aGVHD; B,D,F,H) at day 7, and were incubated with 1 μg/mL LPS or mFasL in the presence or absence of neutralizing anti-murine FasL (αFasL) mAb for 24 hours. The IL-18 concentration in each supernatant was measured by ELISA. Control hamster IgG did not down-regulate the secretion of IL-18 from Kupffer cells from hosts having transplantation with BDF1 or WT B6 cells upon stimulation with mFasL. Data are presented as mean ± SD of triplicate cultures. Similar results were obtained in 3 independent experiments. ND indicates not detectable; NS, not significant.
levels were elevated in the WT hosts having transplantation with caspase-1–deficient donor cells (column B), whereas the inverse combination resulted in only a trace increase in the serum IL-18 concentration (column C). These data indicate that most of the elevated IL-18 in the circulation of aGVHD hosts is derived from hosts. Indeed, Kupfer cells from WT recipients engrafted with WT or caspase-1–deficient donor cells secreted IL-18 upon stimulation with mFasL, whereas those from caspase-1–deficient hosts secreted a trace amount of it, if any (data not shown). Moreover, only minor changes were observed in various target organs for aGVHD in caspase-1–deficient mice engrafted with WT or caspase-1–deficient allografts (H. I., unpublished data, December 2000). Therefore, IL-18 may be released from recipient cells in a FasL-mediated, caspase-1–dependent manner.

This is the first report that demonstrates FasL-mediated IL-18 accumulation in actually occurring diseases. As previously reported, pro–IL-18 is stored in Kupfer cells,22 and IL-18 requires cleavage for its secretion by appropriate enzymes that are distinct depending on the sorts of stimuli.7,13 The molecular basis of the processing of IL-18 is still unknown. A recent study demonstrated that upon stimulation with LPS, caspase-1 is activated via Toll-like receptor (TLR)-4, a signaling receptor for LPS, but independently of myeloid differentiation factor-88, an adaptor molecule for TLR-mediated signaling, leading to IL-18 secretion.22 However, we do not yet know the signaling pathway after TLR-4 activation. This is also the case for FasL-induced IL-18 secretion. Our present study suggests that FasL-mediated IL-18 processing might consist of 2 pathways. One is a caspase-1–like–dependent pathway, and the other is the caspase-1–dependent pathway shown here. We do not know the mechanism for how FasL stimulation selectively activates caspase-1 in a GVHD-induced mice or how the same stimulation preferentially activates caspase-1–like in P acnes–primed mice. Perhaps, FasL-mediated activation of different caspases may be involved in the development of distinct pathophysiologic events, such as massive liver necrosis in P acnes/soluble FasL-treated mice11 or severe bile duct destruction in aGVHD.23 Caspase-1 might be a potential therapeutic target for manipulating aGVHD. We are now investigating the pathologic role of IL-18 in aGVHD.

Acknowledgments

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References

11. Tsutsui H, Kajikai K, Kuida K, et al. Caspase-1-dependent secretion of IL-18 is derived from hosts. WT or caspase-1–deficient BALB/c (Casp1-/-) mice were lethally irradiated and underwent transplantation with WT or caspase-1–deficient B6/129 spleen cells (5×107). At day 5, serum was sampled and IL-18 levels were determined. Serum IL-18 in irradiated WT or caspase-1–deficient BALB/c mice having transplantation with BALB/c spleen cells at day 5 was not detectable. NS indicates not significant. Data represent the mean ± SD of 5 mice in each experimental group. Similar results were obtained in 3 independent experiments.
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