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Skin-Specific Caspase-1-Transgenic Mice Show Cutaneous Apoptosis and Pre-Endotoxin Shock Condition with a High Serum Level of IL-18[1]

Kei-ichi Yamanaka,* Minoru Tanaka,† Hiroko Tsutsui,‡ Thomas S. Kupper,§ Kunihiko Asahi,* Haruki Okamura,‡ Kenji Nakanishi,‡ Misao Suzuki,¶ Nobuhiko Kayagaki,‖ Roy A. Black, Douglas K. Miller,†† Kunio Nakashima,† Masayuki Shimizu,* and Hitoshi Mizutani[2]***

To study the pathophysiological roles of overexpressed caspase-1 (CASP1), originally designated as IL-1β-converting enzyme, we generated transgenic mice in which human CASP1 is overexpressed in their keratinocytes. The transgenic mice spontaneously developed recalcitrant dermatitis and skin ulcers, characterized by the presence of massive keratinocyte apoptosis. The skin of the mice contained the active form of human CASP1 and expressed mRNA for caspase-activated DNase, an effector endonuclease responsible for DNA fragmentation. Their skin and sera showed elevated levels of mature IL-18 and IL-1β, but not of IFN-γ. The plasma from these animals induced IFN-γ production by IL-18-responsive NK cells. Administration of heat-killed *Propionibacterium acnes*, a potent in vivo type 1 cell inducer, caused IFN-γ-mediated lethal liver injury in the transgenic mice, which was completely inhibited by treatment with neutralizing anti-IL-18 Ab. These results indicated that in vivo overexpression of CASP1 caused spontaneous apoptotic tissue injury and rendered mice highly susceptible to exogenous type 1 cell-inducing condition in collaboration with endogenously accumulated proinflammatory cytokines. *The Journal of Immunology,* 2000, 165: 997–1003.

Caspase-1 (CASP1),[3] originally designated as IL-1β-converting enzyme, is a member of the intracellular cysteine protease family (1). CASP1 is believed to be involved in both apoptotic cell death and processing of particular cytokines, including IL-1β. Analyses of CASP1 knockout mice (2) and transgenic mice with dominant negative CASP1 mutations (3) supported these biological roles of CASP1 in vivo. Recently, CASP1 has been shown to have the capacity to cleave pro-IL-18, a biologically inactive precursor of IL-18, into mature IL-18 in vitro (4, 5). IL-18 is a pleiotropic cytokine, produced by activated macrophages (6, 7), that induces IFN-γ production by lymphocytes and up-regulates their functional Fas ligand (FasL) expression (7). IL-18 was originally discovered in the serum of mice sequentially treated with *Propionibacterium acnes* and LPS (6). In fact, CASP1-deficient mice showed impaired responses to *P. acnes* and LPS (4, 5). Serum levels of IL-1β and IL-18 were not elevated in *P. acnes*-primed CASP1-deficient mice after LPS challenge, whereas they were increased in LPS-challenged *P. acnes*-primed controls (5). Because a model of CASP1 overexpression has not been established, the pathophysiological roles of overactivated CASP1 are still unclear. In this study, we generated human CASP1 (hCASP1)-overexpressing transgenic mice, because hCASP1 cleaves not only human IL-1β and pro-IL-18 but also murine homologues (5) and is distinguishable from endogenous murine CASP1. Because ubiquitous overexpression of CASP1 resulted in fetal death, we expressed the gene specifically in basal keratinocytes, an important component of skin. Keratinocytes constitutively produce both pro-IL-1β (8) and pro-IL-18 (9) and lack endogenous CASP1 activity under normal conditions (8), allowing us to investigate whether overactivated CASP1 is involved in systemic facilitation of IL-1β and IL-18 in vivo. The keratinocyte-specific hCASP1-transgenic mice (KCASP1Tg) spontaneously suffered from chronic dermatitis under specific pathogen-free conditions, revealed abnormally elevated serum levels of IL-18 and IL-1β, and were prone to hepatitis by treatment with heat-killed *P. acnes*, which by itself only causes multiple hepatic granulomas but in collaboration with exogenous IL-18 induces severe liver injury (10, 11).

Materials and Methods

DNA construct and transgenic mice

cDNA (1.4 kb) encoding the entire coding region of the human pro-CASP1 was ligated into human keratin 14 promoter (a gift from Dr. E. Fuchs, University of Chicago, Chicago, IL) and rabbit β-globin intron (a gift from Dr. T. Tanaka, Kyoto University, Kyoto, Japan) by blunt-end ligation (Fig. 1). The linear keratin 14/CASP1 DNA fragment was injected into fertilized eggs of C57BL/6 mice (Charles-River Japan, Yokohama, Japan) as reported previously (12). A keratinocyte-specific human CASP1-transgenic mouse line (KCASP1Tg) was established. The offspring were screened for incorporation of the transgene by PCR and Southern blotting analyses using DNA from the tail skin.

Departments of *Dermatology and †Biochemistry, Faculty of Medicine, Mie University, Tsu, Japan; ‡Department of Immunology and Medical Zoology, Hyogo College of Medicine, Nishinomiya, Japan; §Harvard Skin Disease Research Center, Brigham and Women's Hospital, Boston, MA; ¶Center for Animal Resources and Development, Kumamoto University, Kumamoto, Japan; ‖Department of Immunology, Juntendo University School of Medicine, Tokyo, Japan; **Immunex Research and Development Corporation, Seattle, WA; and ††Department of Inflammation Research, Merck Research Laboratories, Rahway, NJ

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[2] Address correspondence and reprint requests to Dr. H. Mizutani, Department of Dermatology, Mie University, Faculty of Medicine, Tsu, Mie 514-8507, Japan. E-mail address: h-mizuta@clin.medic.mie-u.ac.jp

[3] Abbreviations used in this paper: CASP, caspase; hCASP1, human CASP1; CAD, caspase-activated DNase; FasL, Fas ligand; KCASP1Tg, keratinocyte-specific CASP1-transgenic mice; m, murine; rh, recombinant human.

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Treatment with neutralizing anti-FasL mAb

One milligram of neutralizing anti-Fas-L mAb, MFL1, was injected i.p. into KCASP1Tg ($n = 4$) weekly from wk 5 to wk 9 (13). Hamster IgG (Sigma, St. Louis, MO) was used as a control.

Northern blotting analysis

Murine CASP1 and CASP3 cDNA were kind gifts from Dr. M. Miura (Osaka University, Osaka, Japan), and cDNA of caspase-activated DNase (CAD) was a gift from Dr. S. Nagata (Osaka University). Total RNA was extracted from tissues of KCASP1Tg and control mice using aliquots of Isogen reagent (Nippon-gene, Toyama, Japan). For Northern blotting analysis, 10 μg of total RNA were size-fractionated by electrophoresis on 2% formaldehyde/agarose gels. RNA was transferred onto nylon membranes (Immobilon-N, Millipore, Millipore-Japan, Tokyo, Japan) and probed with ^{32}P-labeled corresponding cDNAs encoding human and murine CASP1, CASP3, and CAD, respectively. After hybridization, blots were washed twice with 1× SSC/0.1% SDS at 42°C and twice with 2× SSC/0.1% SDS. Blots were then exposed to x-ray film at −70°C.

Cytokines, cytokine assays, and Abs

Murine recombinant IL-1β was obtained from R&D (Minneapolis, MN). Human pro-IL-1β was prepared as described previously by Immunex (Seattle, WA) (14). Recombinant human IL-1β (OCT-43) and anti-human IL-1β Ab (ANOC205) were provided by Dr. Y. Ohmoto (Otsuka Pharmaceuticals, Tokushima, Japan). IL-1α level was determined using ELISA kits from Endogen (Woburn, MA), and concentrations of IL-1β and IFN-γ were determined using kits from R&D. Biologically activity of IL-18 was determined by IFN-γ-inducing assay using the IL-18-responsive murine NK cells (15). Recombinant murine (m) IL-18, rabbit neutralizing anti-mIL-18 Ab and mIL-18 ELISA kits (6) were provided by Dr. M. Ikeda (Hayashibara Institute, Okayama, Japan). The ELISA could detect IL-18 at 10 to 1000 pg/ml of IL-18.

Immunohistochemistry

Biopsy specimens from transgenic and wild-type mice were fixed with phosphate-buffered formalin for 2 h, followed by preparation for cutting of conventional paraffin sections. For frozen sections, samples were immediately frozen in OCT compound (Miles, Elkhart, IN) and stored at −70°C.

Cryostat sections (5 μm) were fixed in acetone for 5 min at 4°C and incubated with the appropriately diluted primary Abs for 1 h. After washing, the bound primary Ab was visualized using a Vectastain Elite kit (Vector Laboratories, Burlingame, CA) with AEC (Dako-Japan, Kyoto, Japan) as the chromogen, according to the manufacturer's instructions.

TdT-mediated dUTP-biotin nick end labeling

DNA fragmentation of skin biopsy specimens in paraffin sections was examined by TUNEL staining as previously reported (16).

Immunoblotting

Immunoblotting was performed as previously described (8). After removal of DNA and RNA using an Isogen kit, the cell lysates of the epidermal sheets from the transgenic and control mice were suspended in SDS-sample buffer under reducing conditions. The electrophoresed protein was transferred onto a nitrocellulose membrane (Schleicher & Schuell, Dassel, Germany) with a semidry blotter (Bio-Rad, Hercules, CA). The membranes were incubated with primary Ab for 1 h, followed by a second incubation with alkaline phosphatase-conjugated anti-mouse or anti-rabbit IgG Ab and then with Western blue chromogen (Promega, Madison, WI).

Pro-IL-1β processing activity

The freshly prepared epidermal sheet lysate was incubated with recombinant human (rh) proIL-1β for 10 to 30 min; then SDS-sample buffer was added and boiled immediately (14). Samples were analyzed by immunoblotting using anti-hIL-1β Ab (ANOC205) followed by treatment with a Western blue detection kit (Promega).

Induction of liver injury

Heat-killed *P. acnes* (1 mg) was injected i.p. into KCASP1Tg (4 and 10 wk old, 5 each) and wild-type mice to induce hepatitis. To protect against the lethal effects of *P. acnes*, transgenic mice were given 200 μg of neutralizing rabbit anti-IL-18 Ab i.p. (6) for 2 consecutive days before *P. acnes* injection. Thirteen minutes before and 24 h after *P. acnes* injection, 100 μg anti-IL-18 Ab were additionally injected. Rabbit IgG was used as a control.

Results and Discussion

Transgenic mice overexpressed with hCASP1 in their skin

Transgenic mice were generated using the DNA shown in Fig. 1*A*. The offspring were screened for incorporation of the transgene by PCR and Southern blotting analyses using DNA from the tail skin. Of a total of 96 live-born mice, 4 (3 males and 1 female) were transgenic for hCASP1. KCASP1Tg were healthy at birth and grew normally but were smaller than wild-type littermates before wk 8. After this time point, KCASP1Tg manifested chronic active dermatitis. One of three male KCASP1Tg was mated with wild-type females, and generated KCASP1Tg and wild-type offspring in an 1:1 male:female ratio. However, interbreeding of the heterozygous mice generated only wild-type offspring for as yet unknown reasons. All experiments were performed on the line heterozygous for the transgene compared with nontransgenic, wild-type littermates.

Ulcerative desquamation accompanied with keratinocyte apoptosis

From wk 8, KCASP1Tg manifested moderate, focal dermatitis around their eyes, which rapidly developed into severe erosive dermatitis, occasionally showed skin ulcers, and simultaneously extended onto face, ear, neck, trunk, and legs within a few weeks under specific pathogen-free conditions (Fig. 1*B*). Reepithelialization occurred, but erosion and ulcers relapsed. After 16 wk, multiple skin ulcers formed, the ear and the eyelids were deformed, and the facial hair and the extremities were lost, only leaving the integument with multiple scars.

At the light microscopic level, the epidermis of KCASP1Tg did not show particular histological changes by wk 6. The thick epidermis around the ulcers of 10-wk-old KCASP1Tg revealed psoriasis-like changes including parakeratosis (Fig. 1*C*). The dermis of the ulcers was characterized by infiltration of many mononuclear cells. Keratinocytes in the lesions showed eosinophilic necrosis with nuclear condensation, which is a feature of apoptotic keratinocytes. In fact, the skin lesions contained many keratinocytes the nuclei of which were stained positively by TUNEL staining, which identifies fragmented DNA (16) (Fig. 1*D*), whereas no TUNEL-positive keratinocytes were observed in the skin of control littermates (data not shown). High levels of hCASP1 were detected in the thick epidermis of KCASP1Tg (Fig. 1*E*), whereas no hCASP1 was detected in those of controls (data not shown). To investigate whether these ulcers were due to the action of functional FasL induced by mature IL-18 (17) that is processed from the endogenous pro-IL-18 by exogenous hCASP1 (described below), we administered neutralizing anti-murine FasL mAb (13, 18) into KCASP1Tg before the onset of skin changes. However, even after intensive treatment with anti-FasL mAb, skin ulcers developed (data not shown). This suggested that keratinocyte apoptosis might be induced independently of the action of the Fas/FasL pathway. IL-18 did not induce apoptosis in any of the cell lines or primary cultured cells that we tested (our unpublished data). Furthermore, keratinocyte-specific IL-1α transgenic mice did not show apoptotic skin ulcers (19), suggesting that IL-1β, the functions of which are the same as those of IL-1α, might not be involved in skin apoptosis in KCASP1Tg.

Active hCASP1 in the skin

hCASP1 mRNA was observed only in the skin of KCASP1Tg but not in other tissues of these mice, such as the liver, kidney, colon, lung, brain, and spleen (Fig. 2*A*). hCASP1 was not observed in these tissues from wild-type littermates (data not shown). In contrast, endogenous murine CASP1 mRNA was expressed in the

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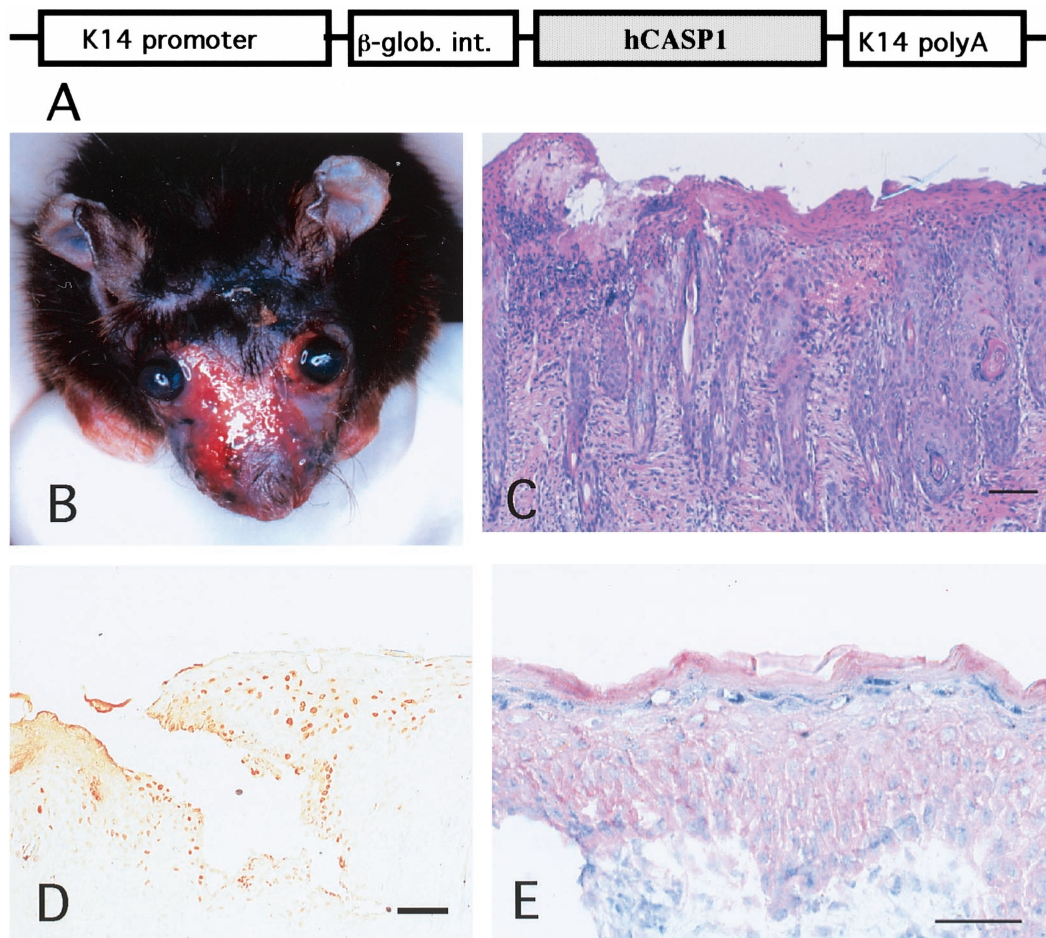

FIGURE 1. Phenotypes of KCASP1Tg. *A*, Schematic structure of the transgene. The human CASP1 cDNA was ligated to the human keratin promoter to drive its basal keratinocyte-specific expression. The transgene construct also contained the rabbit β-globin intron sequence and the keratin 14 (K14) polyadenylation signal to aid in processing of the transcript (45). *B*, Phenotype of KCASP1Tg 18 wk after birth. Erosion started from the face and extended to the ear and neck at the 8th week, and multiple ulcers were formed on the face, trunk, and extremities. Reepithelialization with atrophic skin occurred, but erosion and ulceration quickly relapsed. The hair of the face and eyelids disappeared, and the eyes were covered with a fibrous membrane. *C*, Skin sections from the mice in *B* are shown with hematoxylin-eosin staining. KCASP1Tg epidermis revealed apoptotic cell death with eosinophilic necrosis and marked leukocyte infiltration in the dermis. The epidermis surrounding the ulcers was significantly thicker than nontransgene mouse skin. Bar, 50 μm. *D*, DNA fragmentation was detected in the nuclei of the keratinocytes around the ulcer by TUNEL staining (16). Bar, 50 μm. *E*, Immunoreactive CASP1 was diffusely detected by staining with anti-hCASP1 p20/p10 Ab (G273) (22) in the keratinocytes of the thick KCASP1Tg epidermis. Bar, 50 μm.

latter but not in the skin of both KCASP1Tg and wild-type littermates (data not shown). In general, caspases require appropriate processing to exert their own bioactivities (1). Pro-CASP1 has been reported to undergo autoproteolysis to yield mature CASP1 in vitro (20–22), suggesting that KCASP1Tg might contain spontaneously activated hCASP1 in their skin. To investigate this possibility, we measured the size of hCASP1 protein in the skin lysate from KCASP1Tg by immunoblotting analysis using anti-hCASP1 Ab (22). As shown in Fig. 2*B*, the skin lysate from KCASP1Tg contained both active form components of hCASP1, p20, and p10, and the precursor of p45, while the skin lysate from wild-type littermates contained none of these components, indicating that hCASP1 in the skin of KCASP1Tg is spontaneously cleaved into the mature form.

Next, we investigated whether apoptosis of keratinocytes with active hCASP1 was accompanied with the activation of caspase cascade leading to the activation of effector endonucleases, which is involved in various types of apoptotic cell death. CASP3 is an apoptotic executioner exerting its action commonly after various kinds of apoptosis, including receptor-mediated, such as Fas- and TNF-αR-, and mitochondrial stress-mediated apoptosis (23–26). The KCASP1Tg skin expressed CASP3 mRNA (Fig. 2*C*), whereas skin of control littermates did not. CAD is a recently identified endonuclease that functions downstream of the caspase cascade and directly induces nuclear fragmentation (27, 28). The epidermis of KCASP1Tg expressed CAD mRNA at high levels, whereas CAD mRNA was not detectable in control mouse skin (Fig. 2*D*). Taken together, these observation suggested that apoptotic changes in the skin lesions of KCASP1Tg (Fig. 1*D*) might be associated with activation of the caspase cascade and its downstream endonuclease, CAD.

Next, we tested the processing activity of hCASP1 in the KCASP1Tg skin using pro-IL-1β as a substrate in vitro. The KCASP1Tg epidermis lysate cleaved 31-kDa rhpro-IL-1β into the mature 17-kDa form (Fig. 2*E*). This cleavage was inhibited by a synthetic CASP1 inhibitor (Ac-Tyr-Val-Ala-Asp-CHO) and iodoacetamide (data not shown).

Furthermore, we examined whether hCASP1 in the skin of KCASP1Tg exerted its biological action in vivo. Because keratinocytes constitutively express both IL-1β and IL-18 at the mRNA level (8, 9), we analyzed the sizes of both cytokines at the protein level in the skin. As shown in Fig. 2*F*, skin lysate from

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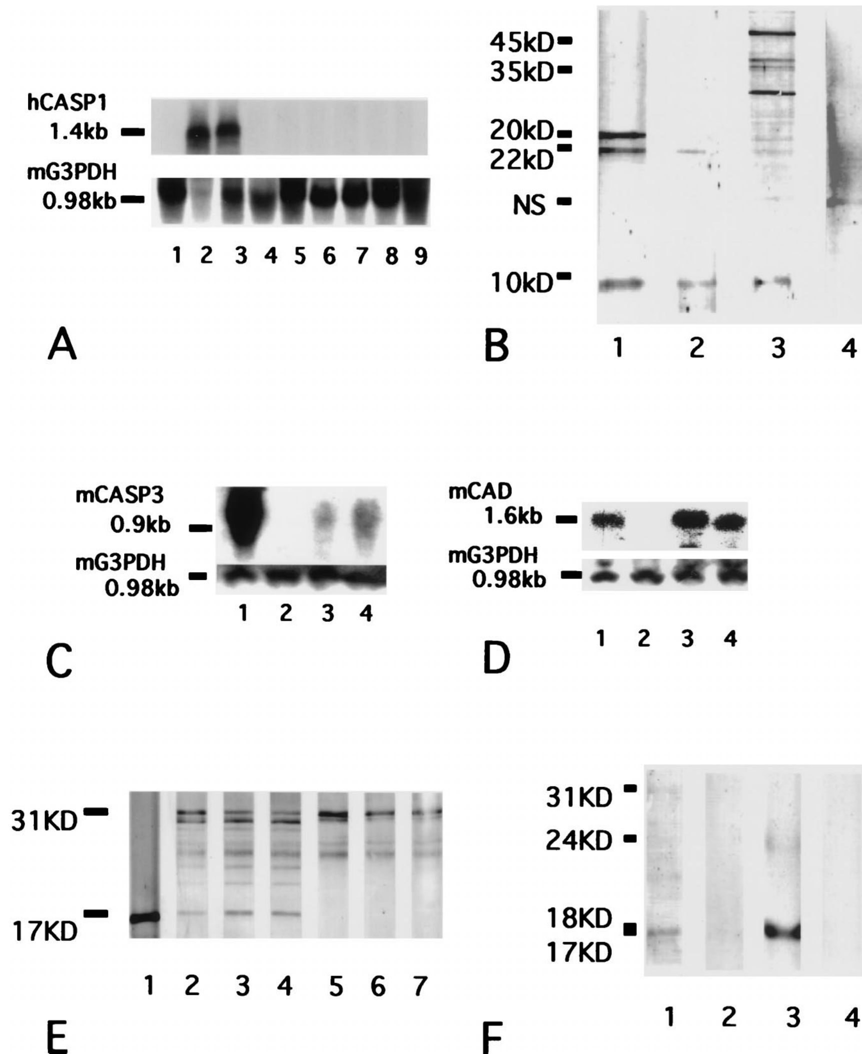

FIGURE 2. Apoptotic skin ulcer in KCASP1Tg. *A*, Northern blotting analysis of skin RNA. No hybridization signals were detected in samples from nontransgenic littermates (*lane 1*). The 1.4-kb hCASP1 mRNA was detected in the ear epidermis (*lane 2*) and back epidermis (*lane 3*), but not in the liver, kidney, colon, lung, brain, or spleen (*lane 4-9*, respectively) from KCASP1Tg. *B*, Immunoblotting analysis of hCASP1 expression in the KCASP1Tg epidermis. Anti-hCASP1 p20/p10 Ab (R105) (22) detected immunoreactive bands of hCASP1 at 22, 20, and 10 kDa in the lysate from positive control THP-1 cells (46) (*lane 1*) and both 20 kDa and 10 kDa in recombinant p20/p10 hCASP1 (*lane 2*) (22). In KCASP1Tg skin, R105 immunoreactive bands were detected at 10, 20, 22, 35, and 45 kDa (*lane 3*), which were not observed in nontransgene controls (*lane 4*). NS, nonspecific band. *C*, Northern blotting analysis of CASP3 mRNA expression. The 0.9-kb CASP3 mRNA was detected in the epidermis of the ear (*lane 3*) and back (*lane 4*) of KCASP1Tg as well as in the thymus (*lane 1*). However, no hybridization signals were detected in skin samples from nontransgenic littermates (*lane 2*). *D*, Northern blotting analysis of CAD mRNA expression. The 1.6-kb CAD mRNA was detected in the epidermis of KCASP1Tg (ear, *lane 3*; back, *lane 4*) as well as the thymus (*lane 1*). However, no hybridization signals were detected in skin samples from nontransgenic littermates (*lane 2*). *E*, The rhpro-IL-1β was incubated with the KCASP1Tg epidermis lysate for the indicated periods, and immunoblotting was performed using polyclonal anti-human IL-1β Ab. The KCASP1Tg epidermis lysate cleaved the rhpro-IL-1β in a time-dependent manner and produced a 17-kDa mature form (*lane 2*, 10 min; *lane 3*, 30 min; *lane 4*, 60 min) that comigrated with rhIL-1β (*lane 1*). Nontransgene epidermis could not cleave rhpro-IL-1β after 10, 30, or 60 min (*lanes 5*, 6, and 7, respectively). *F*, Immunoblotting with polyclonal anti-mIL-1β Ab and anti-mIL-18 Ab. Epidermis from KCASP1Tg back showed two bands corresponding to 31-kDa pro-IL-1β and intense 17-kDa mature IL-1β (*lane 1*). No clear 17-kDa band was detected in the wild-type epidermis (*lane 2*). Epidermis from KCASP1Tg back skin showed two bands, pro-IL-18 (24 kDa) and intense mature IL-18 (18 kDa) (*lane 3*), but wild-type epidermis had no mature IL-18 (*lane 4*).

KCASP1Tg contained both mature IL-18 (18 kDa) and mature IL-1β (17 kDa), whereas that from wild-type littermates expressed none of these molecules, indicating that the transfected hCASP1 spontaneously cleaves endogenous precursors of IL-1β and IL-18 in vivo through its spontaneous activation.

High concentration of IL-18 in the circulation

Next, we investigated whether local activation of IL-18 and IL-1β by exogenous hCASP1 results in systemic accumulation of individual mature cytokines. As shown in Fig. 3*A*, significantly high levels of IL-18 were observed in the sera of KCASP1Tg 4 wk after birth. In contrast, wild-type littermates had low levels of IL-18 in their sera throughout their lives (<0.1 ng/ml). The serum concentration of IL-18 in KCASP1Tg gradually increased with growth. In contrast, a very small amount of IL-1β was detected in the sera of KCASP1Tg only from the age of 12 wk (Fig. 3*A*), which was not observed in those of wild-type littermates. Overactivation of hCASP1 in the keratinocytes appeared to preferentially facilitate serum concentration of IL-18 rather than IL-1β. There are several possible explanations to account for this discrepancy. 1) Keratinocytes might contain larger amounts of pro-IL-18 than pro-IL-1β. In fact, skin lysate from KCASP1Tg contained 30-fold more IL-18 (13.0 ± 3.1 ng/mg skin

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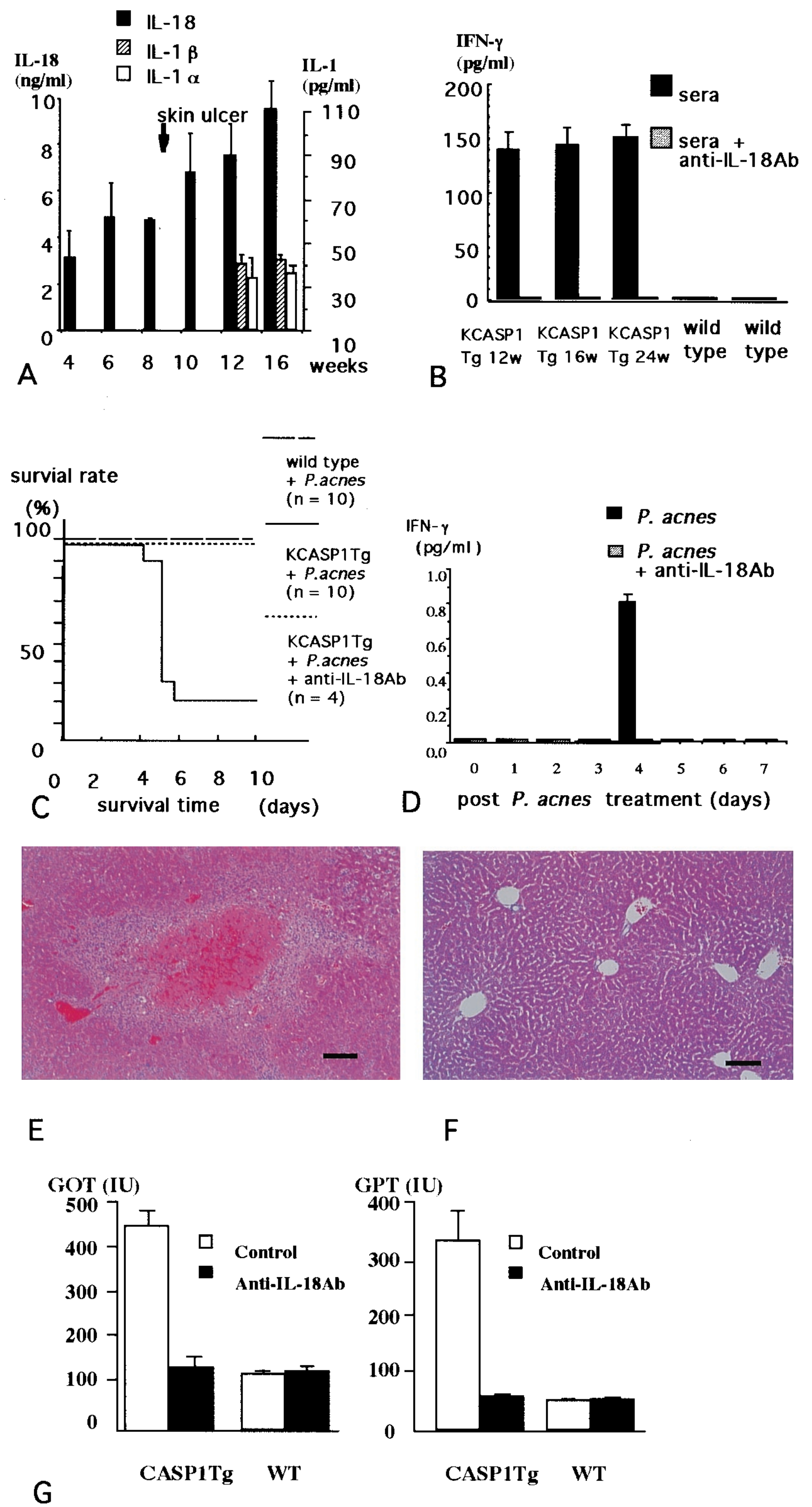

FIGURE 3. KCASP1Tg are highly susceptible to heat-killed *P. acnes*. *A*, The plasma IL-18 level in KCASP1Tg was elevated at wk 4 and increased after development of skin manifestations. Levels of IL-1β and IL-1α were below the limits of detection, but increased after development of the phenotype from 12 wk. None of the three cytokines was detectable in wild-type mice. *B*, Serum samples from KCASP1Tg at 12, 16, and 24 wk old induced IFN-γ production by NK cells. Neutralizing anti-IL-18 Ab completely suppressed IFN-γ production. *C*, Survival curve of wild-type mice (– – – –), KCASP1Tg (——), and KCASP1Tg treated with anti-IL-18 (·····) after *P. acnes* treatment. Neutralizing anti-IL-18 Ab completely inhibited *P. acnes*-induced hepatitis. *D*, serum IFN-γ level of KCASP1Tg reached a peak 4 days after *P. acnes* injection. Anti-IL-18 Ab-treated KCASP1Tg did not show detectable IFN-γ after injection with *P. acnes*. *E* and *F*, Liver specimens from *P. acnes*-treated KCASP1Tg were sampled on day 6 and were fixed with phosphate-buffered formalin followed by staining with hematoxylin and eosin. Severe hepatitis comprised of massive hepatocyte death and granuloma formation was observed (*E*; bar, 100 μm). Liver specimens were obtained from *P. acnes* plus anti-IL-18 Ab-treated mice on day 7 (F; bar, 100 μm). Small granulomatous foci were detected in these specimens, but hepatitis was markedly reduced compared with specimens from control IgG-treated animals. *G*, Alanine transaminase (GPT) and aspartate aminotransferase (GOT) in the sera of the mice were measured simultaneously. Elevation of transaminase were completely inhibited by anti-IL-18 Ab treatment.

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lysate) than IL-1β (578.7 ± 133.5 pg/mg skin lysate). 2) Affinity to hCASP1 might be different between pro-IL-1β and pro-IL-18. Ghayur et al. (5) reported that murine pro-IL-1β requires larger amounts of recombinant hCASP1 for its cleavage than murine pro-IL-18, suggesting that mature pro-IL-18 might competitively inhibit the processing of mature IL-1β. 3) Skin and vessels might be composed of much higher numbers of IL-1R-expressing cells than IL-18R-expressing cells, resulting in effective consumption of mature IL-1β secreted. 4) IL-1β might be more rapidly metabolized than mature IL-18. To confirm that serum IL-18 in KCASP1Tg is the mature form, we examined the biological activity of serum IL-18 in KCASP1Tg and found that sera from KCASP1Tg had the capacity to induce IFN-γ production by IL-18-responsive cloned NK cells (15, 17) (Fig. 3*B*). Furthermore, this IFN-γ-inducing activity was completely inhibited by neutralizing anti-IL-18 Ab, indicating that KCASP1Tg sera contained active IL-18. However, IFN-γ was not detectable in the serum of KCASP1Tg under normal conditions. Thus, KCASP1Tg constitutively secreted mature IL-18 into the circulation.

Development of fatal hepatitis by P. acnes *administration*

Because IL-18 causes acute liver injury in *P. acnes*-primed mice (6, 10, 17), we investigated whether KCASP1Tg were highly susceptible to *P. acnes*. As shown in Fig. 3*C*, >80% of KCASP1Tg died within 6 days after *P. acnes* administration, whereas all of the wild-type littermates were alive and healthy at this time. Serum IFN-γ levels peaked at 4 days after *P. acnes* injection (Fig. 3*D*), whereas IFN-γ was not detectable in *P. acnes*-treated wild-type littermates (data not shown). The administration of anti-IL-18 Ab inhibited the elevation of IFN-γ in *P. acnes*-treated KCASP1Tg (Fig. 3*D*). As expected, *P. acnes*-treated KCASP1Tg manifested subacute liver injury (Fig. 3, *E–G*). Pretreatment with a high level of neutralizing anti-IL-18 Ab completely rescued KCASP1Tg from both *P. acnes*-induced fatal liver injury (Fig. 3, *C, E, F,* and *G*). Treatment with control rabbit IgG did not protect KCASP1Tg from *P. acnes*-induced lethal hepatitis (data not shown). Their susceptibility to *P. acnes*-induced liver injury was not different before and after the onset of skin changes (data not shown). As previously reported, systemic administration of *P. acnes* induces polarization of both hepatic and splenic T cells into type 1 T cells through IL-12 produced endogenously by *P. acnes*-elicited macrophages (29, 30). Although the precise mechanism responsible for the death of KCASP1Tg after *P. acnes* treatment is not clear, severe shrinkage of glomeruli in the kidney was observed, which was also prevented by preadministration of anti-IL-18 Ab. This suggested a critical role of IL-18 in *P. acnes*-induced circulatory dysfunction in KCASP1Tg, presumably in collaboration with *P. acnes*-induced IL-12 (data not shown). Taken together, these observations indicated that a systemic high level of IL-18 is hazardous once it encounters IL-12, which is readily induced by infection with microbes, such as intracellular bacteria, protozoa, and viruses (31–33).

In the early studies using in vitro overexpression system, CASP1 was believed to be directly responsible for apoptotic cell death (34). However, recent investigations utilizing mice deficient in individual apoptosis-relating CASPs that enable us to test physiological roles of the individual target molecules and utilizing a cell-free system that excludes possible contamination of live cell-derived molecules have revealed that CASP1 acts as a processing enzyme for particular cytokines rather than an apoptosis executioner (24, 25, 35). Overexpressed hCASP1 in the skin causes apoptotic cell death accompanied with accumulation of mRNA for CASP3 and CAD (Figs. 1 and 2). However, we have no evidence that active hCASP1 in KCASP1Tg directly stimulates apoptotic cell death pathway. Active IL-18 and/or IL-1β processed by hCASP1 might play a role in skin apoptosis, because both IL-18 and IL-1β are capable of inducing production of TNF-α, a potent apoptosis-inducing factor (24, 36–38).

As previously reported, IL-18 was involved in endotoxin-induced liver injury in *P. acnes*-primed mice (6, 17). Recently, IL-18-deficient mice clearly demonstrated an essential role of IL-18 in endotoxin-induced liver injury (39). As expectedly, KCASP1Tg are highly susceptible to *P. acnes*-induced hepatitis due to IL-18 endogenously processed by hCASP1 (Fig. 3). Furthermore, IL-18 has been reported to be involved in T cell-mediated liver injury. Administration of Con A, a plant-derived T cell mitogen, induced acute fulminant hepatitis through endogenous induction of FasL, a cytocidal molecule for Fas-expressing hepatocytes, and hepatocytotoxic cytokines, such as TNF-α and IFN-γ (40–42). Blockade of IL-18 protects mice from Con A-induced liver injury (43, 44). KCASP1Tg will give us opportunities to investigate the roles of IL-18 in various types of liver injury.

Overexpression of hCASP1 in keratinocytes caused a high serum level of IL-18 presumably through the autoactivation of hCASP1. Most KCASP1Tg died after *P. acnes* treatment, and the surviving mice suffered from subacute liver injury. KCASP1Tg spontaneously manifested erosion and ulcers infiltrated with inflammatory cells in their skin from the age of 8 wk. The severity of dermatitis increased with age. Thus, overactivation of CASP1 caused diseases with a wide spectrum, including skin, liver, and systemic diseases, suggesting that excess activation of CASP1 might be biohazardous. KCASP1Tg is a good model for intrinsic chronic dermatitis and will be useful to develop new therapeutic regimens based on the suppression of CASP1.

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