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*CORRESPONDENCE
Yasutomo Imai,
imai-yas@hyo-med.ac.jp
Nobuo Kanazawa,
nkanazaw@hyo-med.ac.jp

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Deficiency of inflammatory caspases attenuates IL-33–mediated dermatitis

Mami Amaki[1], Teruasa Murata[1], Nobuo Kanazawa[1]* and Yasutomo Imai[1,2]*

[1]Department of Dermatology, Hyogo Medical University, Nishinomiya, Japan, [2]Imai Adult and Pediatric Dermatology Clinic, Osaka, Japan

Interleukin-33 (IL-33) is a nuclear alarmin expressed in epidermal keratinocytes and plays an important role in atopic dermatitis. Environmental stimulation induces extracellular release of IL-33 from the skin. The molecular mechanisms regulating IL-33 release *in vivo* are not well understood. Previous studies using non-cutaneous tissues or *in vitro* systems have suggested that IL-33 release is independent of caspase-1. In this study, we examined the role of caspase-1 in IL-33 release from keratinocytes *in vivo*. Caspase-1 was detected in the nuclei of murine epidermal keratinocytes. After topical stimulation with the hapten 2,4-dinitrofluorobenzene (DNFB), nuclear IL-33 staining in keratinocytes was rapidly lost in wild-type mice. This change was markedly reduced in caspase-1/11–deficient mice. Genetic deletion of caspase-1/11 reduced IL-33–mediated skin inflammation in mice overexpressing IL-33 in keratinocytes, and reduced expression of type 2 cytokines was also observed in the skin. These findings suggest that caspase-1 may play a role in pathogenic IL-33 release in the skin and may control alarmin activity differently across tissues.

KEYWORDS
atopic dermatitis, caspase-1, IL-33, keratinocytes, skin inflammation

Introduction

Interleukin-33 (IL-33), a nuclear alarmin, is highly expressed in the epidermal keratinocytes of patients with atopic dermatitis [1]. Transgenic mice with skin-specific overexpressing IL-33 develop spontaneous atopic dermatitis-like inflammation in an innate lymphoid type 2 cells (ILC2)-dependent manner, consistent with previous findings from our group and others [2, 3]. We have also demonstrated that IL-33 is released extracellularly from the skin *in vivo* in response to cutaneous stimulation [4]; however, the molecular mechanism by which IL-33 is released from keratinocytes remains poorly understood.

IL-33, similar to Interleukin-1 alpha (IL-1α), is stored in the nucleus under steady-state conditions. Although IL-1α is a nuclear cytokine, its extracellular release requires caspase-1 [5]. We therefore hypothesized that caspase-1 may also be involved in the *in vivo* release of IL-33 from keratinocytes.

Recent studies indicate that IL-33 release from airway epithelial cells occurs in a caspase-1–independent manner and involves gasdermin D-mediated pathways [6]. In contrast, we suggest in this study that the mode of IL-33 release from the skin, relevant to cutaneous pathogenicity, may involve on caspase-1.

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Method

Mice

The transgenic mouse line, hK14mIL33tg (IL33tg), which overexpresses murine IL-33 under the control of the human keratin-14 promoter, was generated as previously described [2]. Caspase-1/11 deficient (Casp1/11KO) mice with a C57BL/6 background have been previously described [7]. IL33tg mice were crossed with Casp1/11KO mice to generate IL33tg Casp1/11KO mice. Genotyping of Casp1/11KO mice was performed by PCR using MightyAmp DNA polymerase and the following primers (5′–3′): ICE-Eco3P (extra), ATGGCACACCACAGATATCGG; ICE-Eco5P(WT), GAGACATATAAGGGAGAAGGG; and ICE-NEO-PHIL (neo), TGCTAAAGCGCATGCTCCAGACTG. PCR conditions were 98 °C for 2 min; 35 cycles of 98 °C for 10 s, 60 °C for 15 s, and 68 °C for 1 min. The expected amplicon sizes were approximately 450 bp for the wild-type allele and 250–300 bp for the knockout allele. To confirm insertion of the IL33 transgene into the mouse genome, genotyping was conducted by PCR employing the primer set msF4358 (5′-GGAGGGGGCAAAGTTTTCAGGGTG-3′) and mIL33R5046 (5′-TTTGCAAGGCGGGACCAGGG-3′). All IL33tg mice used in this study were heterozygous (IL33tg$^{+/-}$). Experimental mice were selected based on PCR genotyping to identify IL33tg$^{+/-}$; caspase-1/11$^{-/-}$ mice. All animal studies were conducted in accordance with the International Guiding Principles for Biomedical Research Involving Animals (published by the Council for the International Organization of Medical Science) and were reviewed and approved by the Animal Use and Care Committee of the Hyogo Medical University (Permit Number of IL33tg Casp1/11KO mice: HCM-0572). All mice used in this study were maintained under specific pathogen-free conditions.

Assessment of *in vivo* IL-33 release

An established *in vivo* assay was employed to assess extracellular IL-33 secretion [4, 8]. Mice were challenged by applying 20 μL of 0.5% 2,4-dinitrofluorobenzene (DNFB) dissolved in acetone/olive oil (4:1) to the left ear, whereas the right ear received vehicle alone. After 6 hours, ear skin tissues were processed for immunofluorescence staining for IL-33. Disappearance of nuclear IL-33 staining in the keratinocytes was interpreted as evidence of IL-33 release into the extracellular space.

Tissue staining and immunofluorescence

The excised skin specimens were fixed with 4% (w/v) paraformaldehyde and embedded in paraffin. Paraffin-embedded tissues were sectioned at 4-μm thickness. Deparaffinized sections were stained with hematoxylin and eosin (H&E) for histological evaluation. Immunofluorescence staining for IL-33 was performed as previously described [4]. Briefly, deparaffinized sections were incubated with an affinity-purified rabbit anti-mouse IL-33 polyclonal antibody [4]. Bound primary antibodies were then detected using a biotinylated goat anti-rabbit IgG secondary antibody (Vector Laboratories, Burlingame, CA), followed by streptavidin-conjugated Alexa Fluor 594 (Invitrogen, Carlsbad, CA). Nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI) using ProLong Gold Antifade Mountant with DAPI (Life Technologies, Gaithersburg, MD). Fluorescence images were acquired using a Zeiss LSM780 confocal laser scanning microscope (Carl Zeiss MicroImaging, Thornwood, NY). For caspase-1 staining, tissue sections were deparaffinized and antigen retrieval was performed by heating in 10 mM citrate buffer (pH 6.0). Nonspecific binding was blocked using 1% bovine serum albumin in phosphate-buffered saline. Sections were then incubated with a Cy3-conjugated anti-Caspase-1 P10 polyclonal antibody (bs-0169R-Cy3; Bioss Antibodies, Woburn, MA) diluted 1:200. Cellstain DAPI solution (340-07971, Dojindo Laboratories, Kumamoto, Japan) was used for nuclear counterstaining. Finally, the slides were mounted using the ProLong Gold Antifade Mountant (without DAPI).

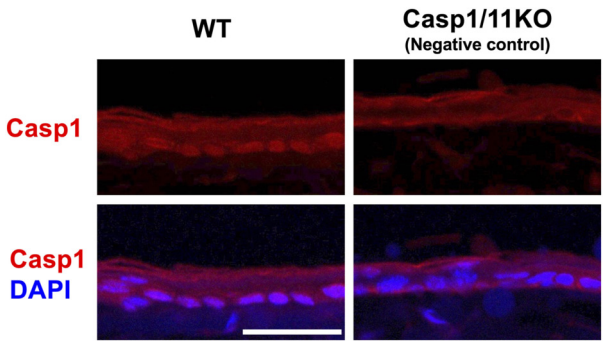

FIGURE 1
Expression of caspase-1 in the epidermis. Immunofluorescence staining of caspase-1 (Casp1) in the epidermis of ear skin from naïve wild-type (WT) and caspase-1/11–deficient (Casp1/11KO) C57BL/6 mice. Cell nuclei were counterstained with 4′,6-diamidino-2-phenylindole (DAPI). Caspase-1 immunoreactivity was prominently detected in the nuclei of epidermal keratinocytes in WT mice, but was absent in Casp1/11 KO mice. Non-specific staining is observed in the stratum corneum, including in Casp1/11 KO mice, indicating that this signal does not represent caspase-1 expression. Scale bar, 50 μm.

Quantitative real-time PCR

A TaqMan RNA-to-Ct Kit (Thermo Fisher Scientific, Waltham, MA) and an ABI7900HT sequence detection system (Applied Biosystems, Foster City, CA) were used for quantitative real-time PCR. Total RNA from mouse skin tissues was isolated using an RNeasy Micro Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Probes for quantitative real-time PCR were obtained from Applied Biosystems Assays-on-Demand (Thermo Fisher Scientific). The numbers of probes for *Il4, Il5, Il13, Il33, Prg2*, and *Gapdh* were Mm00445259_m1, Mm00439646_m1, Mm00434204_m1, Mm00505403_m1, Mm01336479_m1, and Mm99999915_g1, respectively.

Statistical analyses

Statistical analyses were performed using GraphPad Prism 8 software (GraphPad Software, San Diego, CA). Differences were considered statistically significant at $p < 0.05$.

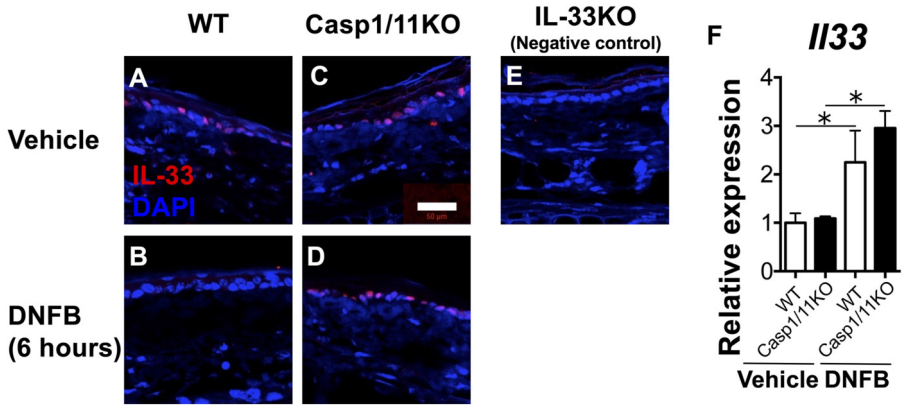

FIGURE 2
Expression of IL-33 in the epidermis after 2,4-dinitrofluorobenzene (DNFB) challenge. Naïve wild type (WT), Caspase-1/11 deficient (Casp1/11KO), or IL-33 deficient (IL-33KO) C57BL/6 mice were challenged with 0.5% DNFB in a 4:1 acetone/olive oil solution on their left ears or with vehicle only on their right ears. **(A–E)** Immunofluorescence staining for IL-33 in the ear skin epidermis. Intense staining of IL-33 is predominantly evident in the nuclei of epidermal keratinocytes **(A,C)**. At 6 h after DNFB challenge, nuclear IL-33 staining in the epidermis is markedly reduced in WT mice **(B)**, whereas IL-33 staining is largely preserved in Casp1/11KO mice **(D)**. Scale bar, 50 μm. *Il33* mRNA expression in the DNFB-treated ear skin Each bar shows *Il33* expression in DNFB-treated or vehicle-treated mouse ears (6 h) **(F)**. Data are expressed as means ± SEM. *$p < 0.05$ (Unpaired t-test). Data are representative of three mice and of two independent experiments.

Results

Mouse caspase-1 is detectable in epidermal keratinocyte nuclei

Caspase-1 localizes in the nuclear or cytoplasmic compartments of certain cell types, including keratinocytes [9]. However, direct evidence of caspase-1 nuclear localization in epidermal keratinocytes *in vivo* remains limited. We performed immunofluorescence staining of mouse skin sections to address this and demonstrated caspase-1 immunoreactivity in the nuclei of epidermal keratinocytes (Figure 1). In contrast, no specific staining was observed in the caspase-1/11–deficient mice, serving as negative controls. These results suggest that caspase-1 localizes to the nuclei of epidermal keratinocytes in mouse skin. We next examined IL-33, another protein known to be stored in keratinocyte nuclei.

Caspase-1/11 is required for DNFB-induced loss of keratinocyte IL-33

IL-33 is constitutively localized in the nuclei of epidermal keratinocytes, where it functions as an alarmin. Loss of nuclear IL-33 staining in keratinocytes was considered an indicator of IL-33 release following DNFB exposure. After topical DNFB application, IL-33 immunoreactivity was markedly decreased in the epidermis (Figures 2A–E). Consistent with our previous report [4], IL-33 fluorescence in the epidermis of wild-type (WT) mice was substantially reduced at 6 h after DNFB treatment (Figures 2A,B).

In contrast, IL-33 immunoreactivity remained detectable in the nuclei of epidermal keratinocytes in caspase-1/11–deficient mice at the same time point (Figures 2C,D).

Previous studies have shown that DNFB-induced loss of IL-33 staining precedes the subsequent upregulation of *Il33* mRNA in the skin [4]. DNFB application increased *Il33* mRNA expression in both wild-type and caspase-1/11–deficient mice (Figure 2F), even though nuclear IL-33 staining remained in knockout mice (Figure 2D). These findings suggest that *Il33* transcription is induced by DNFB independently of IL-33 release.

Thus, caspase-1/11 deficiency prevents DNFB-induced loss of keratinocyte IL-33, prompting us to examine its impact on skin inflammatory responses.

Caspase-1/11 deficiency attenuates IL-33–driven skin inflammation *in vivo*

IL33tg mice spontaneously develop atopic dermatitis-like skin inflammation. IL33tg mice crossed with caspase-1/11–deficient mice (IL33tg Casp1/11KO mice) exhibited markedly attenuated skin inflammation compared with that in IL33tg mice (Figure 3A–D). Histological examination of H&E-stained skin sections (Figure 3E–H) showed pronounced leukocyte and eosinophil infiltration accompanied by epidermal hyperplasia in IL33tg mice (Figure 3G). In contrast, these pathological features were minimal in IL33tg Casp1/11KO mice (Figure 3H), indicating a substantially reduced inflammatory response in the absence of caspase-1/11.

Reduced type 2 cytokine expression in the skin of IL-33 transgenic mice lacking caspase-1/11

Quantitative PCR analysis of the skin tissue showed a significant reduction in the expression of type 2 cytokines, including *Prg2, Il4*, *Il5*, and *Il13*, in IL-33 transgenic mice lacking caspase-1/11 (Figure 4). *Prg2* (encoding eosinophil peroxidase) is a specific marker of murine eosinophils. The observed reduction in *Prg2* expression was consistent with the histological findings from H&E staining, which showed markedly reduced eosinophil infiltration in IL33tg Casp1/11KO mice (Figure 3H) compared with that in IL33tg mice (Figure 3G). These findings indicate that caspase-1/11 deficiency suppresses IL-33–driven type 2 immune responses in the skin.

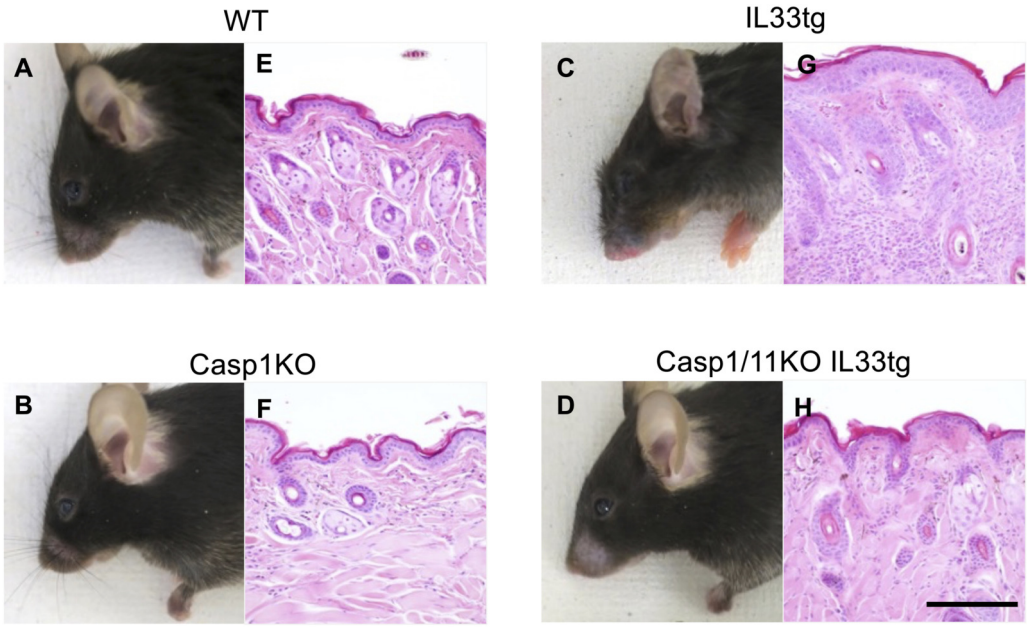

FIGURE 3
Attenuated skin inflammation in caspase-1/11–deficient IL-33 transgenic mice. Cutaneous manifestations **(A–D)** and hematoxylin and eosin (H&E) staining **(E–H)** of ear skin from wild-type (WT), caspase-1/11–deficient (Casp1/11KO), hK14mIL33 transgenic (IL33tg), and caspase-1/11–deficient hK14mIL33 transgenic (IL33tg Casp1/11KO) mice. IL33tg mice showed visible skin changes, including erythema, exudation, crust formation, and/or scaling **(C)**. In contrast, these dermatitis symptoms were less apparent in IL33tg Casp1/11KO mice **(D)**. Consistently, hematoxylin and eosin (H&E)-stained sections from IL33tg mice showed prominent inflammatory cell infiltration **(G)**, whereas inflammatory cell accumulation was reduced in IL33tg Casp1/11 KO mice **(H)**. Images are representative of at least five mice per group (20–25 weeks of age) from two independent experiments. Scale bar, 100 μm.

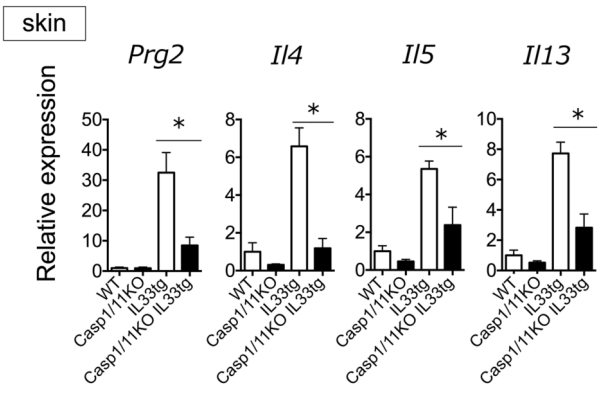

FIGURE 4
Reduced expression of type 2–associated cytokine genes in the skin of caspase-1/11–deficient IL-33 transgenic mice. Expression levels of *Prg2*, *Il4*, *Il5*, and *Il13* in the skin of 8-week-old wild-type (WT), caspase-1/11–deficient (Casp1/11KO), hK14mIL33 transgenic (IL33tg), and caspase-1/11–deficient hK14mIL33 transgenic (IL33tg Casp1/11KO) mice, as determined using quantitative PCR. Data are presented as mean ± SEM (n = 5 per group). $p < 0.05$ (unpaired *t*-test).

Discussion

This study has a limitation related to the genetic model used. The knockout mice employed in this study have historically been described as caspase-1–deficient mice; however, these animals are now recognized to carry a combined deficiency of both caspase-1 and caspase-11 [7, 10]. This occurs because the *Casp1* and *Casp11* genes are in close genomic proximity within the same gene cluster, which results in the simultaneous disruption of both loci in this knockout strain. Because this study used caspase-1/11 double-deficient mice, the potential contribution of caspase-11 to the observed phenotypes cannot be formally excluded. However, a careful examination of previous studies indicates that epithelial inflammasome-related responses, including IL-33 regulation, are mainly mediated by caspase-1 rather than caspase-11. Indeed, caspase-11 is primarily implicated in noncanonical inflammasome signaling in myeloid cells, whereas caspase-1–dependent mechanisms are important in epithelial and tissue-resident cell responses [11]. Because caspase-11 has been reported to localize predominantly in the cytoplasm, its direct involvement in the nuclear export of the nuclear alarmin IL-33 in keratinocytes is considered less likely.

Previous studies concluded that caspase-1 expression was restricted to hematopoietic cells. However, these conclusions were largely based on the absence of detectable interleukin-1 beta–processing activity and the limited sensitivity of detection methods. Subsequent studies using improved methodologies have revealed low-level but functional caspase-1 expression in epithelial cells, including keratinocytes [9]. As IL-33 is a nuclear alarmin, caspase-1 involvement in IL-33 release requires the presence of caspase-1 within keratinocyte nuclei. Therefore, we examined the subcellular localization of caspase-1 and demonstrated the presence of caspase-1 in the nuclei of epidermal keratinocytes (Figure 1).

In this study, the disappearance of nuclear IL-33 staining in keratinocytes was interpreted as an indirect evidence of IL-33

release into the extracellular space. Notably, a similar phenomenon has been reported in experimental models of allergic rhinitis, wherein allergen challenge induces a rapid loss of IL-33 staining in nasal epithelial cells, concomitant with increased IL-33 levels in nasal lavage fluids [8]. These observations support the use of decreased nuclear IL-33 staining as a surrogate marker for extracellular IL-33 release in epithelial tissues. However, we acknowledge that loss of nuclear staining does not directly quantify the extracellular IL-33 levels. Therefore, future studies using biochemical measurements are required.

Currently, no definitive evidence is available to indicate that caspase-1 is required for IL-33 release, and several studies have instead suggested a role for caspase-1 in IL-33 inactivation [12]. Pharmacological inhibition of caspase-1 may not fully reflect its role in IL-33 release, as previous studies have shown that the presence of caspase-1 protein, rather than its enzymatic activity, can be required for the release of nuclear alarmins such as IL-1α [5]. If caspase-1–mediated IL-33 inactivation was essential *in vivo*, IL-33–transgenic mice lacking caspase-1/11 would be expected to demonstrate exacerbated skin inflammation. However, skin inflammation was markedly reduced in these mice. Changes in downstream immune responses, such as ILC2 activation, may also contribute to this phenotype. In addition, because caspase-1/11 was deleted in all cells in the mice we used, we cannot exclude the possibility that caspase-1 deficiency in immune cells influences IL-33–induced skin inflammation. However, the marked reduction in inflammation suggests that caspase-1/11 deficiency may affects an early step in IL-33–driven skin inflammation, likely IL-33 release from keratinocytes. Thus, although IL-33 can be released independently of caspase-1 in other tissues [6], our results suggest that caspase-1 is involved in the pathogenic release of IL-33 from keratinocytes *in vivo*.

Data availability statement

No datasets were generated during this study.

Ethics statement

All animal studies were reviewed and approved by the Animal Use and Care Committee of Hyogo Medical University. This article does not contain any studies with human participants performed by any of the authors.

Author contributions

MA and YI contributed to the study design and manuscript preparation. MA, TM, and YI contributed to the study and animal handling. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declared that generative AI was used in the creation of this manuscript. Generative AI (ChatGPT, OpenAI, and GPT-5.2) was used solely to improve the clarity and grammar of the text. All scientific content, data interpretation, and conclusions were generated by the authors, who carefully reviewed and revised the AI-generated output and assume full responsibility for the accuracy of the final text.

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