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Phospholipase Cε has a crucial role in ultraviolet B-induced neutrophil-associated skin inflammation by regulating the expression of CXCL1/KC

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Phospholipase C (PLC) ε is a phosphoinositide-specific PLC regulated by small GTPases including Ras and Rap. We previously demonstrated that PLCε has an important role in the development of phorbol ester-induced skin inflammation. In this study, we investigated the role of PLCε in ultraviolet (UV) B-induced acute inflammatory reactions in the skin. Wild-type (*PLCε*$^{+/+}$) and *PLCε* gene knockout (*PLCε*$^{-/-}$) mice were irradiated with a single dose of UVB at 1, 2.5, and 10 kJ/m^2 on the dorsal area of the skin, and inflammatory reactions in the skin were histologically evaluated up to 168 h after irradiation. In *PLCε*$^{+/+}$ mice, irradiation with 1 and 2.5 kJ/m^2 UVB resulted in dose-dependent neutrophil infiltration in the epidermis at 24 and 48 h after irradiation. When mice were irradiated with 10 kJ/m^2 of UVB, most mice developed skin ulcers by 48 h and these ulcers became more severe at 168 h. In *PLCε*$^{-/-}$ mice, UVB (1 or 2.5 kJ/m^2)-induced neutrophil infiltration was markedly suppressed compared with *PLCε*$^{+/+}$ mice. The suppression of neutrophil infiltration in *PLCε*$^{-/-}$ mice was accompanied by attenuation of UVB-induced production of CXCL1/keratinocyte-derived chemokine (KC), a potent chemokine for neutrophils, in the whole skin. Cultured epidermal keratinocytes and dermal fibroblasts produced CXCL1/KC in a PLCε-dependent manner after UVB irradiation, and the UVB-induced upregulation of CXCL1/KC in these cells was significantly abolished by a PLC inhibitor. Furthermore, UVB-induced epidermal thickening was noticeably reduced in the skin of *PLCε*$^{-/-}$ mice. These results indicate that PLCε has a crucial role in UVB-induced acute inflammatory reactions such as neutrophil infiltration and epidermal thickening by at least in part regulating the expression of CXCL1/KC in skin cells such as keratinocytes and fibroblasts.

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Solar ultraviolet (UV) radiation induces various acute effects on the skin and most of the harmful biological effects of UV are attributed to UVB (290–320 nm).[1–5] The acute effects of UVB include inflammation, apoptosis, gene mutation, and immunosuppression.[1–4] The inflammation caused by UVB has been well-documented clinically and histologically. Clinically, UVB-induced skin inflammation results in cutaneous injury such as erythema, heat, swelling, and pain.[1,2,6] Histologically, skin inflammation caused by UVB is characterized by dilation of blood vessels,[1,2] infiltration of neutrophils,[1,7–10] and epidermal thickening.[2,9,11–13] Biochemical changes including cytokine expression are also induced during UVB-induced acute skin inflammation.[1] UVB-induced skin inflammatory reactions constitute a potent tumor-promoting event that contributes to photocarcinogenesis.[14–16] Thus, development of a method for regulating UVB-induced acute effects is important for protection against photocarcinogenesis. However, the molecular mechanisms by which UVB induces acute effects on the skin have not been fully clarified.

Phospholipase C (PLC) ε is one of the members of the phosphoinositide-specific PLC family that catalyzes the hydrolysis of a membrane phospholipid, phosphatidylinositol 4,5-bisphosphate, to generate two important second messengers, diacylglycerol and inositol 1,4,5-trisphosphate.[17] Diacylglycerol and inositol 1,4,5-trisphosphate induce the

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activation of protein kinase C (PKC) and mobilization of calcium from intracellular stores, respectively.[18,19] PLCε was identified by us[19,20] and other groups[21,22] as a downstream effector of the Ras family small GTPases. It has been suggested that PLCε mediates diverse signals from outside the cell because of the multiple regulatory mechanisms of the enzyme.[17] Pathophysiological roles of PLCε have been studied in various animal models carrying artificial or spontaneous mutations in the chromosomal gene.[23–25] Also, using positional cloning, the human *PLCε* gene *PLCE1* was identified as a causal gene for nephrotic syndrome.[26] However, few studies on the role of PLCε in external stimuli-induced pathophysiological conditions have been carried out.

We previously demonstrated that PLCε has an important role in the development of phorbol ester-induced skin inflammation.[27] The purpose of this study was to investigate the involvement of PLCε in UVB-induced acute inflammation in the skin using $PLC\varepsilon^{-/-}$ mice. We show that PLCε has a crucial role in UVB-induced neutrophil infiltration in the skin by regulating expression of CXCL1/keratinocyte-derived chemokine (KC), a potent chemokine for neutrophils. We also demonstrate that PLCε regulates UVB-induced epidermal thickening.

MATERIALS AND METHODS

Chemicals

A PLC inhibitor U73122 and a tumor necrosis factor (TNF)-α converting enzyme (TACE) inhibitor TAPI-2 were purchased from Calbiochem (La Jolla, CA, USA) and BIOMOL International (Plymouth Meeting, PA, USA), respectively. Dimethyl dulfoxide (DMSO) was obtained from Nacalai Tesque (Kyoto, Japan).

Mice

$PLC\varepsilon^{-/-}$ mice (129/Sv × C57BL/6 mixed background) were developed as described previously.[28] $PLC\varepsilon^{+/+}$ and $PLC\varepsilon^{-/-}$ mice were obtained by mating with $PLC\varepsilon^{+/-}$ male and $PLC\varepsilon^{+/-}$ female animals. The care and use of the mice was reviewed and approved by the Institutional Animal Committee of Kobe University Graduate School of Medicine. Mice were housed under special pathogen-free conditions and all animal experiments were conducted according to the 'Guideline for Animal Experimentation at Kobe University Graduate School of Medicine'.

UVB Irradiation and Assessment of its effects on Skin

A bank of six TL 20W/12RS fluorescent lamps (Philips, Eindhoven, Holland) was used to irradiate mice.[29] These lamps emit a continuous spectrum from 275 to 390 nm, with a peak emission at 313 nm; ~65% of that radiation is within the UVB wave range. The irradiance was 3.8 J/m^2 per second at a distance of 40 cm as measured by a UVR-305/365D digital radiometer (Tokyo Kogaku Kikai KK, Tokyo, Japan). For the study of UVB-induced skin inflammation, 12–15-week-old mice were selected and divided into two groups ($PLC\varepsilon^{+/+}$ or $PLC\varepsilon^{-/-}$) of 10 mice (5 male mice; 5 female mice). The day before UVB irradiation, dorsal areas of the skin were shaved. Mice were irradiated with a single dose of 1, 2.5, or 10 kJ/m^2 UVB, and biopsied at the indicated time points.

Histology and Immunostaining

Biopsied skins were fixed with 10% neutralized formalin, embedded in paraffin, and then stained with hematoxylin and eosin (H&E) or anti-proliferating cell nuclear antigen (PCNA) antibody (M0879; Dako Cytomation, Copenhagen, Denmark). For statistical studies, the total number of neutrophils in neutrophil microabscesses in the epidermis in each section was counted and the density of neutrophils in the epidermis was expressed as the mean number of neutrophils/100 μm length of epidermis. The thickness of the epidermis (distance from the top of the basement membrane to the bottom of the stratum corneum) and percentage of PCNA-positive cells among total basal cells in the basal cell layer were measured in five fields per sample and averaged.

Identification of Neutrophils

Neutrophils were identified on H&E-stained skin sections. Specifically, pink-stained leukocytes with nuclei definitely divided into two to three lobes were identified as neutrophils. The identification of neutrophils was performed also with immunostaining using anti-Gr-1 antibody (MAB; 1037, R&D Systems, Minneapolis, MN, USA) on frozen sections (Supplementary Figure 1).

Quantitative Real-Time PCR (qRT–PCR)

Total cellular RNA isolation using Trizol (Invitrogen, Carlsbad, CA, USA) and cDNA synthesis were performed as described previously.[27] qRT–PCR was carried out using the SYBR Premix Ex Taq II kit (Takara Bio, Kyoto, Japan) with the Thermal Cycler Dice Real-Time System (Takara Bio). Relative mRNA levels were determined using the comparative *Ct* method followed by normalizing to the β-actin mRNA level in each cDNA sample. The sequence of the primers were 5′-GCTTGTTCAGTTTAAAGATGGTAGGC-3′ and 5′-CGTG TTGACCATACAATATGAAAGACG-3′ for CXCL1/KC, 5′-AG CCCACGTCGTAGCAAACCACCAA-3′ and 5′-ACACCCATT CCCTTCACAGAGCAAT-3′ for TNF-α, and 5′-CTACAATGA GCTGCCTGTGG-3′ and 5′-CAACGTCACACTTCATGATGG-3′ for β-actin.

Primary Cultures of Epidermal Keratinocytes and Dermal Fibroblasts

Primary cultures of epidermal keratinocytes and dermal fibroblasts were performed as described previously[30] with minor modifications. Briefly, epidermal keratinocytes were isolated from the dorsal skin of newborn mice at postnatal day 1 and seeded with defined keratinocyte-SFM supplemented with growth factors onto plastic plates coated with type 1-A collagen (Nitta gelatin, Osaka, Japan). Dermal

fibroblasts were isolated from the dorsal skin of newborn mice at postnatal day 1 and cultured on ordinary culture plates in DMEM supplemented with 10% FBS.

CXCL1/KC Determination by ELISA

CXCL1/KC protein content in the culture medium was measured using a Mouse CXCL1/KC ELISA Kit (Biosource, Camarillo, CA, USA) according to the manufacturer's instruction.

Statistical Analysis

Statistical differences in the data between $PLC\varepsilon^{+/+}$ and $PLC\varepsilon^{-/-}$ mice were determined using Student's *t*-test. $P<0.05$ was considered to be statistically significant.

RESULTS

UVB Induces Neutrophil infiltration in the Skin

As an initial step of investigating whether PLCε has a role in UVB-induced acute skin inflammation, we irradiated the skin of $PLC\varepsilon^{+/+}$ and $PLC\varepsilon^{-/-}$ mice with a single dose of UVB at 1 kJ/m^2, and examined neutrophil infiltration in the skin as a representative UVB-induced acute inflammatory reaction at 0, 3, 6, 12, 18, 24, 48, and 168 h after irradiation (Figure 1). Before UVB irradiation, no neutrophil infiltration was detected in the skin of either $PLC\varepsilon^{+/+}$ or $PLC\varepsilon^{-/-}$ mice (Figures 1a and g). When mice were irradiated with 1 kJ/m^2 of UVB, neutrophils were first observed at 12 h mainly in the blood vessels located in the subcutaneous fat tissue of $PLC\varepsilon^{+/+}$ mice (Figure 1b). Neutrophil infiltration became

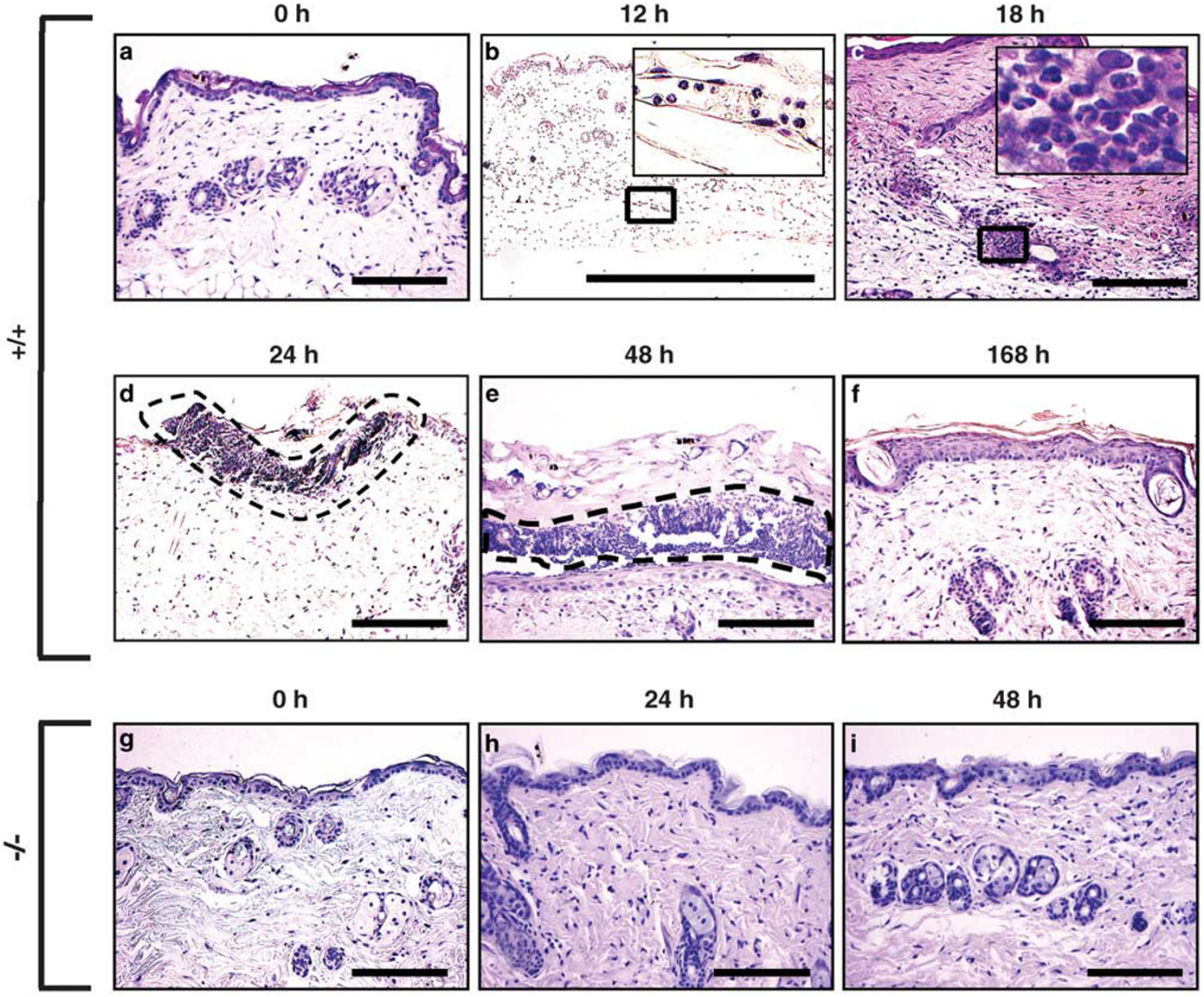


Figure 1 Histological findings of the time course of neutrophil infiltration in the epidermis of $PLC\varepsilon^{+/+}$ and $PLC\varepsilon^{-/-}$ mice irradiated with 1 kJ/m^2 of UVB. The shaved dorsal skins of $PLC\varepsilon^{+/+}$ and $PLC\varepsilon^{-/-}$ mice were irradiated with a single dose of UVB at 1 kJ/m^2 ($n=10$ for each *PLCε* background, 5 male mice and 5 female mice). Skin specimens were prepared at 0, 12, 18, 24, 48, and 168 h after irradiation, and were stained with H&E. Representative photographs of skin sections from $PLC\varepsilon^{+/+}$ (+/+) and $PLC\varepsilon^{-/-}$ (−/−) mice are shown. Insets in (**b**) and (**c**) show a blood vessel containing neutrophils and neutrophil infiltration in the stroma of the dermis, respectively. Neutrophil microabscesses are surrounded by dotted lines in (**d**) and (**e**). Bar, 100 μm.

more severe at 18 h and neutrophils were present not only in blood vessels but also in the dermal stroma (Figure 1c). No apparent neutrophil infiltration was found in the skins of $PLC\varepsilon^{+/+}$ mice collected at 3 or 6 h after irradiation (data not shown). In skin sections of $PLC\varepsilon^{+/+}$ mice collected at 24 and 48 h, infiltration of neutrophils was present in the epidermis, most of which resulted in the formation of patchy neutrophil microabscesses (Figures 1d and e; Supplementary Figure 1). Neutrophil infiltration in the epidermis disappeared almost completely by 168 h in $PLC\varepsilon^{+/+}$ mice (Figure 1f). Although neutrophil infiltration was observed in the dermis at 24 and 48 h in $PLC\varepsilon^{+/+}$ mice, the extent of the infiltration was much less than that in the epidermis. In contrast, in $PLC\varepsilon^{-/-}$ mice, no neutrophil microabscesses were found in the epidermis at any time point examined (Figures 1h and i).

UVB-Induced Neutrophil Infiltration in the Skin is Suppressed in $PLC\varepsilon^{-/-}$ Mice

We further assessed the effect of higher UVB doses, 2.5 and 10 kJ/m^2, on neutrophil infiltration in the skins of $PLC\varepsilon^{+/+}$ and $PLC\varepsilon^{-/-}$ mice at 24 and 48 h. When mice were irradiated with 2.5 kJ/m^2 of UVB, the $PLC\varepsilon^{+/+}$ mice and some $PLC\varepsilon^{-/-}$ mice showed epidermal neutrophil microabscess(es) at 24 and/or 48 h. When mice were irradiated with 10 kJ/m^2 of UVB, most mice developed skin ulcers by 48 h irrespective of the *PLCε* background and these ulcers became more severe at 168 h (data not shown). After irradiation with 1 and 2.5 kJ/m^2 of UVB, the density of infiltrated neutrophils in $PLC\varepsilon^{-/-}$ mice was significantly lower than in $PLC\varepsilon^{+/+}$ mice at 24 and 48 h (Figure 2). Based on these results, 1 kJ/m^2 was considered to be a suitable UVB dose for investigating the difference in UVB-induced skin inflammation between $PLC\varepsilon^{+/+}$ and $PLC\varepsilon^{-/-}$ mice, and was used in all further studies.

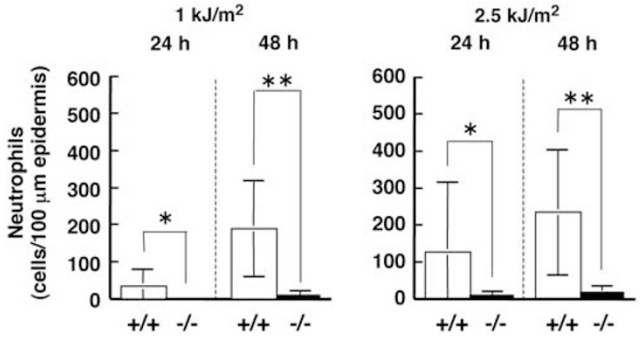


Figure 2 Comparison of the density of neutrophils in the epidermis of $PLC\varepsilon^{+/+}$ and $PLC\varepsilon^{-/-}$ mice irradiated with UVB. The shaved dorsal skins of $PLC\varepsilon^{+/+}$ (white bar) and $PLC\varepsilon^{-/-}$ (black bar) mice were irradiated with a single dose of UVB at 2.5 kJ/m^2 ($n = 10$ for each *PLCε* background, 5 male mice and 5 female mice). Skin specimens were prepared at 24 and 48 h after irradiation, and were stained with H&E. The density of neutrophils in the epidermis at each time point was calculated together with samples used in Figure 1 as described in Materials and methods. Data are expressed as mean ± s.d. $^*P < 0.05$; $^{**}P < 0.01$.

UVB-Induced Production of CXCL1/KC is Attenuated in the Skin of $PLC\varepsilon^{-/-}$ Mice

We next investigated the cause of attenuated neutrophil infiltration in UVB-treated $PLC\varepsilon^{-/-}$ mice skin. The possibility that attenuated neutrophil infiltration in $PLC\varepsilon^{-/-}$ mice is due to a reduction in PLCε-dependent chemotaxis in neutrophils was ruled out because the cells do not express PLCε.[27] Infiltration of neutrophils in the inflammatory site is controlled by chemokines.[31,32] It has been shown that, among the various chemokines, CXCL8/interleukin (IL)-8 and CXCL1/KC, a functional mouse homolog of human CXCL8/IL-8, have a pivotal role in the mediation of UVB-induced inflammation in the human skin.[10,33–35] Thus, we examined the UVB-induced changes in CXCL1/KC expression in the skin of $PLC\varepsilon^{+/+}$ and $PLC\varepsilon^{-/-}$ mice by qRT–PCR (Figure 3a). Similar results were obtained at UVB doses of 1.0 and 2.5 kJ/m^2. In $PLC\varepsilon^{+/+}$ mice, UVB irradiation resulted in an increase in CXCL1/KC mRNA expression up to 24 h. In contrast, in the skin of $PLC\varepsilon^{-/-}$ mice, CXCL1/KC mRNA levels at each time point were much lower than those in $PLC\varepsilon^{+/+}$ mice, although expression of CXCL1/KC mRNA was also enhanced by UVB irradiation in the skin. To identify skin cell type(s) that produce CXCL1/KC in response to UVB cultured keratinocytes and fibroblasts were irradiated with UVB and protein levels of CXCL1/KC in culture supernatants were analyzed by ELISA (Figure 3b). The levels of CXCL1/KC in the supernatants of non-irradiated keratinocytes and fibroblasts from $PLC\varepsilon^{+/+}$ mice were significantly higher than those from $PLC\varepsilon^{-/-}$ mice. UVB irradiation resulted in an increase in this chemokine in the supernatants of both cell types irrespective of *PLCε* genotypes and the levels of CXCL1/KC in the supernatants of both of the cell types from $PLC\varepsilon^{+/+}$ mice were much higher than those from $PLC\varepsilon^{-/-}$ mice. Evidence for the involvement of PLCε in the UVB-induced production of CXCL1/KC in keratinocytes and fibroblasts was further supported by the observation that a broad spectrum PLC inhibitor U73122 significantly suppressed the UVB-induced upregulation of CXCL1/KC in these cells from $PLC\varepsilon^{+/+}$ mice (Figure 3c). It has been demonstrated that UVB is a potent stimulator of TNF-α production in the skin.[36] Also, TNF-α is known to induce CXCL1/KC production in some cell types.[37] These reports raise the possibility that UVB upregulates CXCL1/KC production through enhancing TNF-α production in skin cells. Thus, we examined the possible involvement of TNF-α in UVB-induced CXCL1/KC production. No alteration in the level of TNF-α mRNA in the skin after UVB irradiation between $PLC\varepsilon^{+/+}$ and $PLC\varepsilon^{-/-}$ mice was observed (Figure 4a). In addition, a TACE inhibitor, TAPI-2, did not affect the levels of CXCL1/KC in the supernatants of either keratinocytes or fibroblasts from $PLC\varepsilon^{+/+}$ mice after UVB irradiation (Figure 4b). Thus, an indirect effect of UVB through TNF-α on CXCL1/KC production was ruled out.

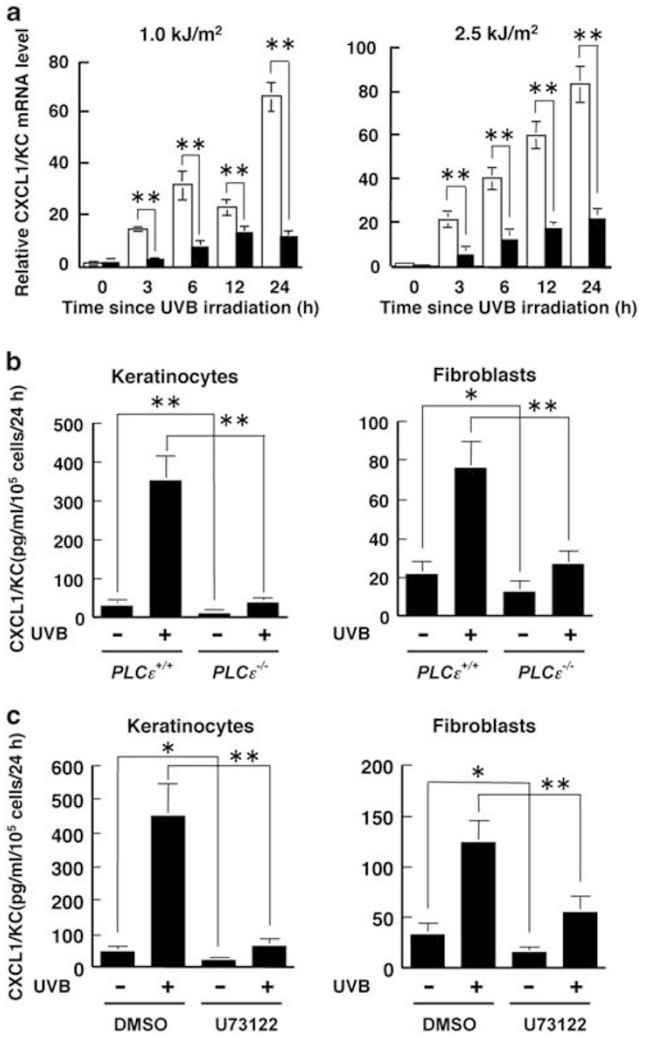


Figure 3 UVB-induced expression of CXCL1/KC in the skin of $PLC\varepsilon^{+/+}$ and $PLC\varepsilon^{-/-}$ mice. (**a**) The shaved dorsal skins of $PLC\varepsilon^{+/+}$ (white bar) and $PLC\varepsilon^{-/-}$ (black bar) mice were irradiated with a single dose of UVB at either 1 or 2.5 kJ/m^2. The CXCL/1KC mRNA level in the skin collected at 0, 3, 6, 12, and 24 h was quantitated by qRT–PCR. The results are expressed as the fold change using the value of the non-irradiated skin as 1. $^{**}P < 0.01$. (**b**) Cultured keratinocytes and fibroblasts from were irradiated with a single dose of UVB at 0.1 kJ/m^2 followed by culturing for another 24 h. Medium was removed before the UVB treatment. CXCL1/KC secretion in the supernatants was quantitated by ELISA and was calculated as pg/ml/10^5 cells/24 h. (**c**) After pre-treatment with 2.5 μM U73122 or 0.1% DMSO for 10 min, cultured keratinocytes and fibroblasts from $PLC\varepsilon^{+/+}$ mice were irradiated with a single dose of UVB at 1 kJ/m^2 followed by culturing for another 24 h in the presence of either 2.5 μM U73122 or 0.1% DMSO. CXCL1/KC secretion in the supernatants was quantitated by ELISA and was calculated as pg/ml/10^5 cells/24 h. The results shown are representative of three independent experiments.

UVB-Induced Epidermal Thickening is Reduced in the Skin of $PLC\varepsilon^{-/-}$ Mice

We next investigated the effect of PLCε deficiency on the UVB-induced proliferative response of the epidermis. At 168 h after irradiation with 1 kJ/m^2 of UVB, $PLC\varepsilon^{+/+}$ mouse skin showed a marked increase in epidermal thickness (Figure 1f, Figures 5a and b). UVB-induced epidermal thickening was significantly suppressed in $PLC\varepsilon^{-/-}$ mice. In non-irradiated epidermis, proliferating cells positive for PCNA constituted 3–4% of basal cells in the basal cell layer of the epidermis, and there was no apparent difference in the number of PCNA-positive cells between $PLC\varepsilon^{+/+}$ and $PLC\varepsilon^{-/-}$ mice.[28] UVB irradiation noticeably increased the number of PCNA-positive cells in the basal cells in $PLC\varepsilon^{+/+}$ mice, and the percentage of PCNA-positive cells among the basal cells reached ~60% at 168 h after irradiation (Figure 5b). In contrast, the UVB-induced increase in the number of PCNA-positive cells was markedly lower at ~10% in $PLC\varepsilon^{-/-}$ mice.

DISCUSSION

In the present study, we have shown that the extent of UVB-induced neutrophil infiltration in the mouse skin is closely associated with the level of expression of CXCL1/KC in the skin. Furthermore, our study identified a novel function of PLCε as a critical molecule regulating UVB-induced CXCL1/KC production. Thus, it is considered that UVB-induced neutrophil infiltration is regulated at least in part by PLCε-mediated CXCL1/KC expression. Furthermore, the UVB-induced proliferative response in the epidermis is suppressed in $PLC\varepsilon^{-/-}$ mice. Collectively, our results demonstrate that UVB-activated PLCε has a crucial role in UVB-induced acute inflammatory reactions.

The molecular mechanism of UVB-induced PLCε activation and the downstream signaling pathways from PLCε to neutrophil infiltration in the epidermis and epidermal thickening remain to be elucidated. PLCε is activated by a variety of mechanisms via multiple upstream regulators including GTPases such as Ras, Rap1, Rap2,[17,20–22] and RhoA.[17,20–22] Thus, further investigation of the interaction between UVB and these regulators is necessary to reveal the exact mechanism of UVB-induced PLCε activation. As activated PLCε produces the PKC activator, diacylglycerol, it is possible that PLCε stimulates UVB-induced epidermal neutrophil infiltration and epidermal thickening through a PKC-mediated mechanism. It has been shown that activation of PKCα promotes neutrophil infiltration in the epidermis.[38,39] Also, activation of PKCε[40] and PKCμ[41] has been demonstrated to increase keratinocyte growth.

UVB irradiation at 1.0 and 2.5 kJ/m^2 induced upregulation of CXCL1/KC in the skins of the mice. It has been demonstrated that the depth of UVB (1.25–4 kJ/m^2) penetration in the mouse dorsal skin is the upper dermis.[42] Thus, the UVB-induced increase in CXCL1/KC mRNA in Figure 3a, at least in the case of UVB at 2.5 kJ/m^2, was considered to reflect the result of the mRNA from UVB-irradiated epidermis and dermis. In addition, cultured keratinocytes and dermal fibroblasts responded to UVB to produce CXCL1/KC in our study. These results suggest that UVB directly acts on at least

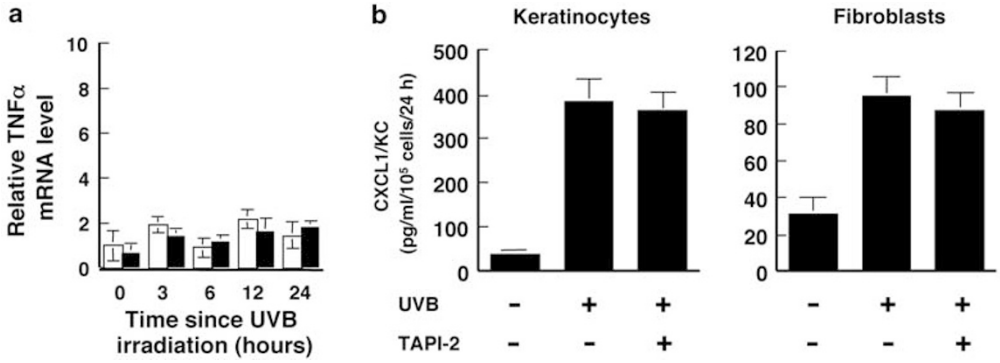
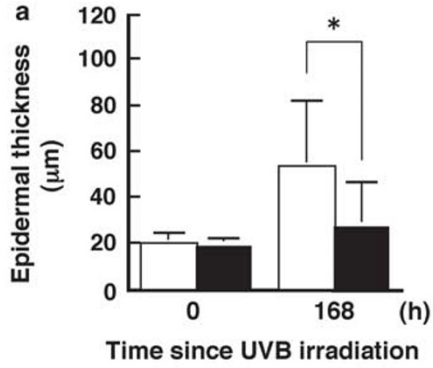


Figure 4 Role of TNF-α in UVB-induced expression of CXCL1/KC in skin cells. (**a**) The shaved dorsal skins of $PLC\varepsilon^{+/+}$ (white bar) and $PLC\varepsilon^{-/-}$ (black bar) mice were irradiated with a single dose of UVB at 1 kJ/m^2. The TNF-α mRNA level in the skin collected at 0, 3, 6, 12, and 24 h was quantitated by qRT–PCR. The results are expressed as the fold change using the value of the non-irradiated skin as 1. (**b**) After pretreatment without or with 200 nM TAPI-2 for 1 h, cultured keratinocytes and fibroblasts from $PLC\varepsilon^{+/+}$ mice were irradiated with a single dose of UVB at 1 kJ/m^2 followed by culturing for another 24 h in the absence or presence of 200 nM TAPI-2. CXCL1/KC secretion in the supernatants was quantitated by ELISA and was calculated as pg/ml/10^5 cells/24 h. The results shown are representative of three independent experiments.



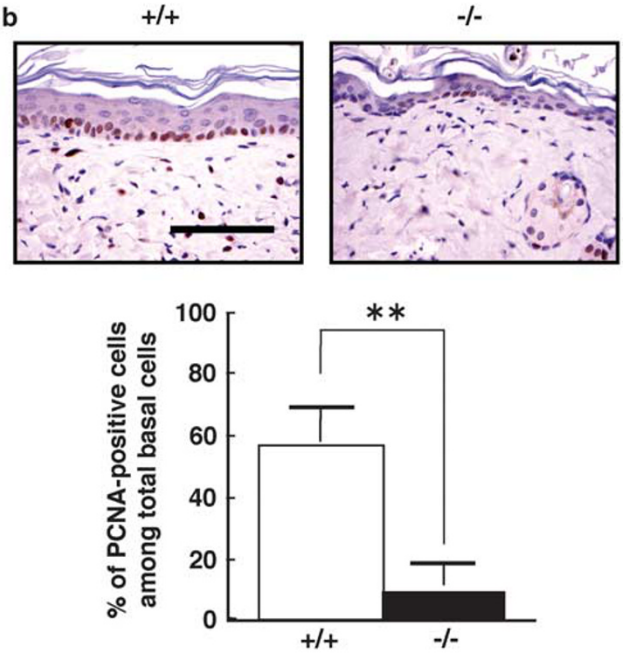


Figure 5 Suppression of the UVB-induced proliferative response in $PLC\varepsilon^{-/-}$ mice. (**a**) The epidermal thickness of $PLC\varepsilon^{+/+}$ (white bar) and $PLC\varepsilon^{-/-}$ (black bar) mice at 0 and 168 h after irradiation with a single dose of UVB at 1 kJ/m^2 used in Figure 1 was calculated as described in materials and methods. Data are expressed as mean ± s.d. $^*P<0.05$. (**b**) The skin sections used in (**a**) were stained with the anti-PCNA antibody. Representative photographs of skin sections from $PLC\varepsilon^{+/+}$ (+/+) and $PLC\varepsilon^{-/-}$ (−/−) mice at 168 h after irradiation are shown (upper two panels). Bar, 100 μm. The percentage of PCNA-positive cells in the basal cell layer against total basal cells in skin sections from $PLC\varepsilon^{+/+}$ (+/+) and $PLC\varepsilon^{-/-}$ (−/−) mouse was determined as described in materials and methods and is expressed as mean ± s.d. (lower panel). $^{**}P<0.01$.

keratinocytes and fibroblasts in the mouse skin to produce CXCL1/KC.

Neutrophil infiltration is observed in a number of other human skin diseases besides UVB-induced skin, including psoriasis[43] and neutrophilc dermatoses.[44] It is well recognized that IL-8-mediated neutrophil infiltration modifies the inflammation process in psoriasis,[43] and it has been shown that CXCL1/KC is involved in psoriasis-like skin inflammation in an animal model.[45] We previously showed that IL-8 is overexpressed in the skin of patients with pyoderma gangrenosum, a representative of neutrophilc dermatoses.[46] We also showed that overexpression of IL-8 using adenovirus vector in human skin grafted on severe combined immunodeficiency mice resulted in neutrophil-associated skin ulcer resembling pyoderma gangrenosum.[46] These findings indicate that IL-8-induced neutrophil infiltration has a role either directly or indirectly in the pathogenesis of several inflammatory skin diseases. As IL-8 is not produced in normal conditions, an increased amount of IL-8 in these diseases must result from dysregulation of IL-8 production. Our findings identify a novel function for PLCε as a critical

molecule regulating UVB-induced neutrophil infiltration in the skin by at least in part regulating the expression of CXCL1/KC in skin cells such as keratinocytes and fibroblasts. It is important to investigate whether PLCε activation and its link to the production of CXCL8/IL-8 also has a role in these human skin diseases.

Supplementary Information accompanies the paper on the Laboratory Investigation website (http://www.laboratoryinvestigation.org)

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DISCLOSURE/CONFLICT OF INTEREST

The authors declare no conflict of interest.

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