Journal of Investigative Dermatology (2010) 130(8):2010-2016.

Acrosyringium Is the Main Site of the Vesicle/Pustule Formation in Palmoplantar Pustulosis

Murakami, Masamoto ; Ohtake, Takaaki ; Horibe, Yoshimune ; Ishida-Yamamoto, Akemi ; Morhenn, Vera B. ; Gallo, Richard L. ; Iizuka, Hajime

Acrosyringium is the main site of the vesicle/pustule formation in palmoplantar pustulosis

Masamoto Murakami[1], Takaaki Ohtake[2], Yoshimune Horibe[3], Akemi Ishida-Yamamoto[1], Vera B. Morhenn[4], Richard L. Gallo[4], and Hajime Iizuka[1]

Departments of [1]Dermatology and [2]Internal medicine, Asahikawa Medical College, Asahikawa, Japan,

[3]Division of Pathology, Daido Hospital, Nagoya, Japan,

[4]Division of Dermatology, University of California San Diego, and VA San Diego Healthcare Center, San Diego, California, U.S.A.

The work area: Asahikawa, Japan

Corresponding: Dr. Masamoto Murakami,

Department of Dermatology, Asahikawa Medical College, Hokkaido 078-8510, Japan

e-mail: mamuraka@asahikawa-med.ac.jp

phone: 0166-68-2523, fax: 0168-68-2529

Short running head: sweat disorganization in PPP

Abbreviations: PPP, palmoplantar pustulosis; GCDFP, gross cystic disease fluid protein; EMA, epithelial membrane antigen; hCAP, human cathelicidin antimicrobial peptide; IL-8, interleukin-8; H.&E., hematoxilin and eosin; DDW, double distilled water; PMN, polymorphonuclear leukocyte; TFA, trifluoroacetic acid; R.T., room temperature; PVDF, polyvinylidene difluoride; TBS, tris buffered saline; TTBS, tween TBS; PBS, phosphate buffered saline; RT-PCR, reverse transcription polymerase chain reaction

KEY words: pustulosis, PPP, cathelicidin, dermcidin, eccrine, sweat

Manuscript information:

Text: 3122 words (Abstract: 190 words), tables: 0, figures: 6

Conflicts of interest: none

Abstract

Pustulosis palmaris et plantaris or palmoplantar pustulosis (PPP) is a refractory pustular eruption on the palms and soles with unknown etiology. Numerous eccrine sweat pores exist on the palms and soles, suggesting the involvement of eccrine sweating in the pathogenesis of PPP. To the best of our knowledge, however, no definite abnormality in sweating has been documented in PPP. Accordingly, we analyzed the eccrine sweat duct involvement in the mechanism of vesicle formation in PPP. Dermatoscopy showed that PPP vesicles are located on the top of the ridges but not in the furrows. The sweat secretion in the lesional area was much lower than that in the non-lesional area with or without pain stimulation to induce sweating. Immunostaining of horizontal sections of the lesions using antibodies against GCDFP-15 and EMA showed that these markers were localized in the cells lining the intraepidermal vesicles. Although the sweat antimicrobial peptides, dermcidin and hCAP-18/LL37, were detected in the fluid of the vesicles/pustules, neither dermcidin nor hCAP-18/LL-37 were over-expressed by neighboring keratinocytes. These findings suggest that the acrosyringium may be involved as the main site of the vesicle formation in the pathomechanism of PPP.

Introduction

Pustulosis palmaris et plantaris or palmoplantar pustulosis (PPP) is a chronic pustular dermatitis characterized by palmoplantar intraepidermal vesicles filled with neutrophils (Uehara and Ofuji, 1974). Though it is a common skin disease often recalcitrant to available treatments, the pathogenesis remains unknown. Numerous eccrine sweat pores exist on the palms and soles, and several reports suggest the involvement of eccrine sweating in the pathogenesis of PPP (Eriksson et al., 1998; Krieg et al., 1992). To the best of our knowledge, however, no definite abnormality in sweating has been documented in PPP.

The eccrine sweat gland is a secretory as well as an excretory organ. Although the sole function of sweat has been considered to be thermoregulation during exposure to a hot environment or during physical exercise, recent evidence indicates that sweat plays a role in the innate immune response. Previously, two major classes of antimicrobial peptides were identified in mammalian skin: cathelicidins (Gallo et al., 1997; Nizet et al., 2001; Zanetti et al., 1995) and ß –defensins (Ali et al., 2001; Harder et al., 1997; Stolzenberg et al., 1997). Cathelicidins, that like many other antimicrobial peptides, are synthesized as a preproprotein (Zanetti et al., 1995). The only cathelicidin in humans is hCAP-18/LL-37 (Agerberth et al., 1995; Frohm et al., 1997a) and is expressed in leukocytes as well as on a variety of epithelial surfaces. The hCAP-18/LL-37 has been detected in human skin keratinocytes, but only at the site of inflammation, suggesting that this antimicrobial peptide functions primarily in response to injury rather than modulating colonization of the skin's surface (Frohm et al., 1997a). Another antimicrobial peptide, dermcidin, recently was detected in the human sweat gland, suggesting that sweat may

play an additional important role in protection against various bacteria (Schittek et al., 2001). Recently, we have demonstrated the expression of hCAP-18/LL-37 in eccrine sweat glands (Murakami et al., 2002), which confirms the role of antimicrobial peptides in protecting the body surface through innate immunity.

In order to dissect the involvement of the eccrine sweat duct in the pathomechanism of vesicle formation in PPP, a possible abnormality in the process of sweating was analyzed in early lesions of PPP. Because the vesicle formation can be rapidly induced by infection, stress, etc., it is plausible to speculate that the preexisting acrosyringium is involved in the formation of the lesion. Our data provide a support for the hypothesis that an abnormality in sweating initiates the formation of vesicles suggesting a pathophysiological mechanism for PPP.

Results

PPP vesicles were located on the ridges of palmar skin

Dermatoscopy clearly demonstrated that small vesicles and vesico-pustules were located in a linear fashion on the top of the ridges but not in the furrows of the palmar skin (Fig 1). Some large vesicles or pustules extended into the furrows.

Sweat secretion in the lesional area was decreased compared to that in the non-lesional area

Sweat secretion was compared between the lesional and non-lesional areas of the palmar skin. At the time of measurement, the patient's palm showed several small vesicles with

a few pustules. Sweat volume in the lesional area (B) was much lower than that in the non-lesional area (A) or the contralateral healthy palm(s) (Fig 2). The sweat volume in the non-lesional area was increased following pain stimulation, while that in the lesional area remained low.

Small vesicles were located in/and around the epidermal sweat ducts in the early lesions

PPP vesicles were located within the epidermis, with typical pustule formation (Fig 3). Keratinous material in the acrosyringium was also observed (Fig 3a). A small vesicle related to acrosyringium could be observed in the epidermis (Fig 3b). The vesicle turned into a tiny pustule filled with small round cells and neutrophils (Fig 3c). Microabscess formation was seen in the epidermis with remarkable inflammatory cell infiltration around the pustule (Fig 3d). Finally, the lesion resolved to normal appearing epidermis (Fig 3e). Using horizontal sections, immunostaining with GCDFP-15 and EMA showed that these signals were localized to the acrosyringium showing pores in the epidermis and the lining cells of the intraepidermal vesicles (Fig 4).

Expression of dermcidin and hCAP-18/LL-37 was not increased in the keratinocytes around the vesicle formation

Dermcidin and hCAP-18/LL-37 were detected in the sweat duct in the stratum corneum in addition to the dermal eccrine sweat ducts (Fig 5). However, neither dermcidin nor hCAP-18/LL37 was overexpressed in the keratinocytes around the vesicles

(Fig 5). The hCAP-18/LL-37 was abundantly expressed in the PMNs in the pustules but dermcidin was not detected (data not shown).

The expression of dermcidin and hCAP-18/LL-37 in PPP vesicles/pustules

The sweat antimicrobial peptides, dermcidin and hCAP-18/LL-37, were detected in the PPP vesicles/pustules (Fig6). Dot-blots performed for the detection of dermcidin showed positive signals in the PPP vesicles/pustules. The hCAP-18/LL-37 western-blots showed three bands (18kd; full-length, 15kd; intermediate-sized, and 4.5kD; mature form), as was already reported previously in sweat (Murakami et al., 2002).

The hCAP-18/LL-37 mRNA was not up-regulated in lesional PMNs

PMNs are known to contain hCAP-18/LL-37. In order to dissect the origin of hCAP-18/LL-37 proteins, LL-37 mRNA expression in L- and B-PMNs were analyzed with both RT-PCR and real-time PCR (Fig 6c). Both L- and B-PMNs contained hCAP-18/LL-37 mRNA. However, there was no significant difference in the mRNA expression levels between L- and B-PMNs as assessed by real-time PCR (Fig 6d).

Discussion

The etiology of PPP remains unknown, and whether PPP and localized pustular psoriasis are distinct entities is still controversial (Burns et al., 2004; Freedberg et al., 2003). However, typical PPP usually presents in patients who do not have a personal or family history of psoriasis. Furthermore, the absence of immunogenetic associations of PPP

with psoriasis suggests that PPP may represent a separate and distinct entity (Burns et al., 2004; Freedberg et al., 2003). Our dermatoscopic findings as well as our documentation of reduced local sweat volume in the lesional area(s) are consistent with the notion that the acrosyringium specifically is involved in the pathogenesis of PPP(Eriksson et al., 1998). GCDFP-15, a conventional marker for the apocrine gland, also cross reacts with the eccrine glands (Viacava et al., 1998). Our results document that the lining cells of the vesicles are positive for GCDFP-15 in addition to EMA, suggesting that the main component of the vesicles could be enlarged or destroyed acrosyringium.

Several histopathological studies regarding the origin of PPP have been published (Uehara and Ofuji, 1974). Briefly, the first phase of this disease is characterized by spongiosis or epidermal vesicle formation followed by the purulent eruption during the second phase. The pustulation begins, when direct contact between the blister fluid and the stratum corneum occurs (Uehara and Ofuji, 1974).

Investigations of the mechanism of the subcorneal sterile pustules formation have focused on the chemotactic factors for PMNs (Bruch-Gerharz et al., 1996; Gillitzer et al., 1996; Kulke et al., 1996; Ozawa et al., 2005), which include IL-8 and components of complement (Anttila et al., 1992; Ozawa et al., 2005). Immunohistochemical studies showed that intracellular epidermal IL-8 was consistently detected in PPP as well as in psoriasis but not in non-lesional or healthy control skin. HuMab 10F8, a novel human mAb against IL-8, significantly reduced clinical disease activity of PPP by inhibiting the formation of fresh pustules (Skov et al., 2008). C3b was also detected on the subcorneal keratinocyte cell membranes (Ozawa et al., 2005), suggesting that complement is also responsible for the intraepidermal migration of PMNs (Ozawa et al., 2005).

Recent evidence indicates skin antimicrobial peptides are also chemotactic for PMNs (Sommer *et al.*, 2000). The peptides, cathelicidin (Bals et al., 1998; Frohm et al., 1997a; Gallo et al., 1997), defensins (Ali et al., 2001), and dermcidin (Schittek et al., 2001) are essential elements of the epithelial defense system participating in a variety of immune mechanisms in both a direct and indirect manner (Gallo et al., 2002; Zasloff, 2002). For human sweat glands, the constitutive expression of dermcidin and inducible expression of cathelicidin have been documented (Murakami et al., 2002; Schittek et al., 2001). Using these peptides as a marker for sweat, we obtained the evidence that the PPP patients had an abnormality of sweat secretion.

Dot-blot and western-blot analyses showed that the vesicle and pustule fluid contains the same antimicrobial peptides found in sweat. It has been reported that dermcidin is continuously secreted in the eccrine sweat but is not induced during inflammation (Rieg et al., 2004; Schittek et al., 2001), while cathelicidins are induced under inflammatory conditions such as psoriasis and wound healing (Frohm et al., 1997b; Gallo et al., 2002). It is known that the source of cathelicidin is the granules of the PMNs (Sorensen et al., 1997; Sorensen et al., 2001). Therefore, we investigated whether the cathelicidin also comes from the surrounding keratinocytes of the lesion. Both dermcidin and hCAP-18/LL-37 were documented in the sweat duct in the stratum corneum in addition to the eccrine sweat ducts in the dermis, as reported previously (Murakami et al., 2002; Schittek et al., 2001). However, no dermcidin or hCAP-18/LL-37 over-expressions were detected in keratinocytes around the vesicles. This suggests that neither dermcidin nor hCAP-18/LL-37 in the fluid came from the surrounding keratinocytes. Quantitative real-time PCR also disclosed no significant difference in the mRNA

expression levels between lesional PMNs and blood PMNs. Based on these findings, we conclude that cathelicidin in the vesicle fluid originated mainly from the sweat-derived contents of the acrosyringium.

Eriksson et al (Eriksson et al., 1998) recently performed a clinical and immunohistological study of PPP, and proposed that the acrosyringium was possibly involved in this disease. This hypothesis was based on the observation that the epidermal eccrine duct structure was not present in any of the specimens from PPP, while it was present in all of the control specimens (Eriksson et al., 1998). By contrast, our results clearly demonstrate the involvement of the acrosyringium in the PPP vesicle formation suggesting that the disease originates from the acrosyringium. However, it is still unclear why the acrosyringium is involved at the initiation of this process, and whether external pathogens are involved in the etiology of this disease. These issues remain to be clarified in order to dissect the pathomechanism of PPP in the future studies.

Materials and Methods

PPP volunteers

After obtaining approval from the ethics committee of Asahikawa Medical College, fifteen volunteer patients (female 13 and male 2, mean age: 62.7±18.5 years; range: 33-82,) with 2-10 yr history of PPP were recruited for this study with informed consent. They had not been treated with any medications before. These patients were diagnosed by at least two board-certified dermatologists according to the clinical features. After

obtaining informed consent, fifteen samples for biopsy and 5 samples for blood and lesional vesicle/pustule samples were collected.

Dermatoscopy

Patients' palms and soles were examined by a dermatoscope (Derma 9500C, KOS Medical K. K., kawakawa, Japan). The pictures were recorded and stored as digital files. Dermatoscopy work was completed by a dermatologist and then checked by another board certified dermatologist.

Sweat measurement

In order to see if a sweat secretion problem existed on the lesional area of the skin, palmar sweat volume was measured with a portable sweat meter TS-1 (TECHNO SCIENCE, Tokyo, Japan), and was compared to non-lesional areas. Probe (1 cm^2) of the sweat meter was applied on the target skin area, and the 2 min sweat volume was measured at both lesional and non-lesional areas on the palms of the PPP patients. In order to see the increase in the pain-induced sweating, the total 2 min sweat volume after pin-prick stimulation was also measured in both lesional and non-lesional areas. The measurement was repeated three times on the same patient.

Tissue and vesicular fluid sampling

Excisional biopsies (about 10 mm size) were taken from palmar lesions of 15 PPP cases with informed consent. The specimens were fixed in 10% buffered formalin overnight

and were embedded in paraffin blocks for routine pathological diagnosis. The 4µm sections were prepared for H.&E., as well as for immunostaining. Horizontal slices of 5 µm thickness were also prepared for additional immunostaining to distinguish the acrosyringium (*vide infra*).

Using a micropipetter, the fluid obtained from the vesicles or vesico-pustules was collected from 5 patients immediately after cutting the top off the lesion and put into a 1.5 ml centrifuge tube. Several vesicles in the area of the lesion were collected and mixed together. One ml of PBS was added and the sample was centrifuged at 15,000 rpm. for 5 min., and spun down. The supernatant was then lyophilized with a Speed Vac overnight, and the pellet was resuspended in 50µl of double distilled water (DDW) for western-blot and dot-blot analyses. All samples were then stored at –80˚C until use.

Lesional polymorphonuclear leukocytes (PMN) collection from the lesion

For western-blot and real time PCR analyses, PMN from palmar pustules (L-PMNs) were collected from 5 patients. The cells were obtained from the lesion with a micropipette after a small incision into the pustule. Collected L-PMNs were washed with PBS 3 times and then re-suspended in 100µl of PBS. 4 ml of blood was drawn using venipuncture. From the blood, PMN (B-PMNs) were isolated and then processed with Polymorphprep (AXIS-SHIELD PoC AS, Oslo, Norway) according to the manufacture's instruction. The samples were re-suspended in 100µl of PBS. All samples were stored at –80˚C until use for RNA and protein extractions.

Antibodies

Rabbit polyclonal antibody to LL-37 used for immunostaining was developed against full-length LL-37(Murakami et al., 2005). Mouse monoclonal antibody to dermcidin (G-18, Santa Cruz Biotechnology, INC., CA), mouse monoclonal antibody to epithelial membrane antigen (EMA; clone E29, DAKO, Denmark), and mouse monoclonal antibody to gross cystic disease fluid protein-15 (GCDFP-15(BRST-2, D6), Signet laboratories, Inc, Dedham, MA) were purchased.

Synthetic peptides for LL-37 and dermcidin

Synthetic LL-37 was commercially prepared by Peptide institute. Inc., Osaka, Japan, and synthetic dermcidin and its precursor Y-P30(Schittek *et al.*, 2001) as a negative control were prepared by Synpep Corp., Dublin, CA. Peptide amino acid sequences were LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES for LL-37, SSLLEKGLDGAKKAVGGLGKLGKDAVEDLESVGKGAVHDVKDVLDSVL for dermcidin, and YDPEAASAPGSGNPCHEASAAQKENAGEDP for Y-P30. The synthetic peptides were purified to over 95% by HPLC and their identity was confirmed by mass spectrometry, respectively.

Western-blot analysis

Protein extraction from PMN samples was performed by acidification with 1% TFA/1M HCL. Briefly, 500µl of the 1% TFA/1M HCl solution was added to the PMNs samples, then vortexed well to destroy the cells, and incubated at room temperature (R.T.) for 30 min. After centrifuging, the supernatant was collected, concentrated by lyophilization, and resuspended in 50 µl of DDW. All samples were stored at –30˚C until use. Protein

samples from PMNs and the vesicle/pustule fluids (10 µl) were separated by 16% Tricine gel (Invitrogen, Carlsbad, CA), respectively, and then transferred onto a PVDF membrane (immobilone-P, Millipore). Ten nmol of LL-37 (as a positive control) and dermcidin (as a negative control) synthetic peptides were also applied, respectively. The membrane was treated with blocking solution (0.1% TTBS: 5% nonfat milk in 0.1% Tween 20 / tris buffered saline (TBS: 150 mM NaCl, 10 mM Tris Base, pH 7.4)) for 60 min at R.T., and then rabbit anti-LL37 polyclonal antibody (1:5000 in the blocking solution) was incubated with the membrane overnight at 4ºC. After washing the membrane 3 times with 0.1% TTBS, horseradish peroxidase labeled goat anti-rabbit IgG polyclonal antibody (1:5000 in the blocking solution, Bio-Rad Laboratorides, Hercules, CA) was incubated with the membrane for 60 min, at R.T. After washing the membrane with 0.1% TTBS, the membrane was immersed in ECL solution (Western Lightning Chemiluminescence Reagents Plus, New Lifescience Products, Boston, MA) for 60 sec, and was then visualized with LAS-3000 imaging system (Fujifilm corp., JAPAN) according to the manufacture's instruction.

Dot-blot analysis

Dermcidin expression in the PPP fluid was evaluated by dot-blot analysis. Briefly, 1µl of fluid samples from the PPP patient was applied onto a nitrocellulose membrane with 32 nmol of dermcidin. The Y-P30 and LL-37 synthetic peptides were used as negative controls. After air-drying the membrane, the immunoreaction was visualized as described above with anti-dermcidin antibody in the blocking solution overnight at 4˚C. After washing the membrane with 0.1% TTBS, a horseradish peroxidase labeled goat

anti-mouse IgM polyclonal antibody (1:5000 in the blocking solution, Santa Cruz Biotechnology, INC., CA) was applied as the second antibody for 60 min at R.T. After washing with 0.1% TTBS, the membrane was immersed in ECL solution for 60 sec and was then visualized with LAS-3000 imaging system.

Immunohistochemistry

Tissue sections were immersed in PBS after deparaffinization and endogenous peroxidase activity was blocked by incubating with 0.3% H_2O_2 in methanol for 30 min. After washing with PBS, the immunostaining with rabbit anti-LL-37 (1:100) and mouse anti-dermcidin (1:100) antibodies was performed using Histofine SAB-PO kits (Nichirei Biosciences Inc, Tokyo, Japan) according to the manufacture's instruction. To visualize the sweat pore structure, horizontal slices were obtained and stainied with mouse anti EMA (1:100) and GCDFP-15 antibodies (1:100). As a negative control, the polyclonal antibody and monoclonal antibodies were replaced by normal rabbit and mouse preimmune IgG diluted with PBS containing 3% BSA at the same protein concentration used for the primary antibodies. All procedures were carried out at R.T. except for the first antibody incubation (4°C, over night).

Reverse transcription-polymerase chain reaction (RT-PCR) and real-time quantitative PCR

Total RNA was extracted from PMNs using RNeasy Mini kit (Qiagen, Valencia, CA), and cDNA was prepared from 1μg of total RNA using iScript cDNA synthesis kit (BIO-

RAD laboratories, Hercules, CA) according to the manufacture's instruction. PCR amplification of LL-37 was performed with the forward primer (5'-GATAACAAGAGATTTGCCCTGCTG -3') and the reverse primer (5'-TTTCTCAGAGCCCAGAAGCCTG -3') for a 173 bp product. Amplification of 18S rRNA was done in parallel for all the samples with QuantumRNA classic 18S internal standard kit (Ambion, Woodward Austin, TX). PCR protocol was denaturation at 90°C for 10 min, followed by 35 cycles of amplification with denaturation at 94°C for 30 sec, primer annealing at 50°C for 30 sec, and extension at 72°C for 1 min. Following the RT-PCR, real-time quantitative PCR was performed using a LightCycler 2.0 system (Roche) to compare the mRNA expression levels. RT reaction was performed in 2µl of DNA Master SYBR Green I (Roche) and 2 µl of each 5 µM primer as described above. Amplification of GAPDH was done in parallel for all samples with LightCycler-primer set for Human GAPDH (Roche Diagnostics, Manheim, Germany). Thermal profile: 95 degree 10 sec, 40x (94°C 15 sec, 60°C 1 min). Results were analyzed using the Comparative Ct Method, where Ct indicates the number of cycles required to reach an arbitrary threshold (Dorschner et al., 2003).

Statistical analysis

The data of the sweat measurement and the real-time PCR were evaluated by STATFLEX software (ver. 4.2, ARTEC inc, Osaka, Japan). Non-parametric *t*-test (Mann-Whitney U test) was applied to compare differences between lesion and blood PMNs. $P<0.05$ was considered to be significant.

References

Agerberth B, Gunne H, Odeberg J, Kogner P, Boman HG, Gudmundsson GH (1995) FALL-39, a putative human peptide antibiotic, is cysteine-free and expressed in bone marrow and testis. *Proc Natl Acad Sci U S A* 92:195-9.

Ali RS, Falconer A, Ikram M, Bissett CE, Cerio R, Quinn AG (2001) Expression of the peptide antibiotics human beta defensin-1 and human beta defensin-2 in normal human skin. *J Invest Dermatol* 117:106-11.

Anttila HS, Reitamo S, Erkko P, Ceska M, Moser B, Baggiolini M (1992) Interleukin-8 immunoreactivity in the skin of healthy subjects and patients with palmoplantar pustulosis and psoriasis. *J Invest Dermatol* 98:96-101.

Bals R, Wang X, Zasloff M, Wilson JM (1998) The peptide antibiotic LL-37/hCAP-18 is expressed in epithelia of the human lung where it has broad antimicrobial activity at the airway surface. *Proc Natl Acad Sci U S A* 95:9541-6.

Bruch-Gerharz D, Fehsel K, Suschek C, Michel G, Ruzicka T, Kolb-Bachofen V (1996) A proinflammatory activity of interleukin 8 in human skin: expression of the inducible nitric oxide synthase in psoriatic lesions and cultured keratinocytes. *J Exp Med* 184:2007-12.

Burns T, Breathnach S, Cox N, Griffiths C (2004) *Rook's Textbook of Dermatology*, 7th edn. Blackwell Publishing: Massachusets.

Dorschner RA, Lin KH, Murakami M, Gallo RL (2003) Neonatal skin in mice and humans expresses increased levels of antimicrobial peptides: innate immunity during development of the adaptive response. *Pediatr Res* 53:566-72.

Eriksson MO, Hagforsen E, Lundin IP, Michaelsson G (1998) Palmoplantar pustulosis: a clinical and immunohistological study. *Br J Dermatol* 138:390-8.

Freedberg I, Eisen AZ, Wolff K, Austen KF, Goldsmith LA, Katz SI (2003) *Fitzpatrick's Dermatology in General Medicine*, 6th edn. The McCraw-Hill Companies: New York.

Frohm M, Agerberth B, Ahangari G, Stahle-Backdahl M, Liden S, Wigzell H, *et al.* (1997a) The expression of the gene coding for the antibacterial peptide LL-37 is induced in human keratinocytes during inflammatory disorders. *J Biol Chem* 272:15258-63.

Frohm M, Agerberth B, Ahangari G, Stahle-Backdahl M, Liden S, Wigzell H, *et al.* (1997b) The expression of the gene coding for the antibacterial peptide LL-37 is induced in human keratinocytes during inflammatory disorders. *J Biol Chem* 272:15258-63.

Gallo RL, Kim KJ, Bernfield M, Kozak CA, Zanetti M, Merluzzi L, *et al.* (1997) Identification of CRAMP, a cathelin-related antimicrobial peptide expressed in the embryonic and adult mouse. *J Biol Chem* 272:13088-93.

Gallo RL, Murakami M, Ohtake T, Zaiou M (2002) Biology and clinical relevance of naturally occurring antimicrobial peptides. *J Allergy Clin Immunol* 110:823-31.

Gillitzer R, Ritter U, Spandau U, Goebeler M, Brocker EB (1996) Differential expression of GRO-alpha and IL-8 mRNA in psoriasis: a model for neutrophil migration and accumulation in vivo. *J Invest Dermatol* 107:778-82.

Harder J, Bartels J, Christophers E, Schroder JM (1997) A peptide antibiotic from human skin. *Nature* 387:861.

Krieg PH, Bacharach-Buhles M, el-Gammal S, Altmeyer P (1992) The pustule in palmoplantar psoriasis: transformed vesicle or mature microabscess? A three-dimensional study. *Dermatology* 185:104-12.

Kulke R, Todt-Pingel I, Rademacher D, Rowert J, Schroder JM, Christophers E (1996) Co-localized overexpression of GRO-alpha and IL-8 mRNA is restricted to the suprapapillary layers of psoriatic lesions. *J Invest Dermatol* 106:526-30.

Murakami M, Dorschner RA, Stern LJ, Lin KH, Gallo RL (2005) Expression and secretion of cathelicidin antimicrobial peptides in murine mammary glands and human milk. *Pediatr Res* 57:10-5.

Murakami M, Ohtake T, Dorschner RA, Schittek B, Garbe C, Gallo RL (2002) Cathelicidin anti-microbial peptide expression in sweat, an innate defense system for the skin. *J Invest Dermatol* 119:1090-5.

Nizet V, Ohtake T, Lauth X, Trowbridge J, Rudisill J, Dorschner RA, *et al.* (2001) Innate antimicrobial peptide protects the skin from invasive bacterial infection. *Nature* 414:454-7.

Ozawa M, Terui T, Tagami H (2005) Localization of IL-8 and complement components in lesional skin of psoriasis vulgaris and pustulosis palmaris et plantaris. *Dermatology* 211:249-55.

Rieg S, Garbe C, Sauer B, Kalbacher H, Schittek B (2004) Dermcidin is constitutively produced by eccrine sweat glands and is not induced in epidermal cells under inflammatory skin conditions. *Br J Dermatol* 151:534-9.

Schittek B, Hipfel R, Sauer B, Bauer J, Kalbacher H, Stevanovic S, *et al.* (2001) Dermcidin: a novel human antibiotic peptide secreted by sweat glands. *Nat Immunol* 2:1133-7.

Skov L, Beurskens FJ, Zachariae CO, Reitamo S, Teeling J, Satijn D, *et al.* (2008) IL-8 as antibody therapeutic target in inflammatory diseases: reduction of clinical activity in palmoplantar pustulosis. *J Immunol* 181:669-79.

Sommer S, Wilkinson SM, Merchant WJ, Goulden V (2000) Sweet's syndrome presenting as palmoplantar pustulosis. *J Am Acad Dermatol* 42:332-4.

Sorensen O, Cowland JB, Askaa J, Borregaard N (1997) An ELISA for hCAP-18, the cathelicidin present in human neutrophils and plasma. *J Immunol Methods* 206:53-9.

Sorensen OE, Follin P, Johnsen AH, Calafat J, Tjabringa GS, Hiemstra PS, *et al.* (2001) Human cathelicidin, hCAP-18, is processed to the antimicrobial peptide LL-37 by extracellular cleavage with proteinase 3. *Blood* 97:3951-9.

Stolzenberg ED, Anderson GM, Ackermann MR, Whitlock RH, Zasloff M (1997) Epithelial antibiotic induced in states of disease. *Proc Natl Acad Sci U S A* 94:8686-90.

Uehara M, Ofuji S (1974) The morphogenesis of pustulosis palmaris et plantaris. *Arch Dermatol* 109:518-20.

Viacava P, Naccarato AG, Bevilacqua G (1998) Spectrum of GCDFP-15 expression in human fetal and adult normal tissues. *Virchows Arch* 432:255-60.

Zanetti M, Gennaro R, Romeo D (1995) Cathelicidins: a novel protein family with a common proregion and a variable C-terminal antimicrobial domain. *FEBS Lett* 374:1-5.

Zasloff M (2002) Antimicrobial peptides of multicellular organism. *Nature* 415:389-95.

Figure Legends

Figure 1.

Dermatoscopic findings of the palm of PPP

The palm of the patient with PPP shows small vesicles, small pustules, desquamation, and erythema (a). Dermatoscopy shows that many vesicles of various sizes are located on the top of the ridge together with large pustules (b).

Figure 2

Lesional area shows decreased sweating compared to the non-lesional area

Two min sweat volume was measured from the non-lesional (A) and lesional area of PPP (B) as well as from a healthy palm (a). Basal sweat secretion of the lesional area was much lower than that of the non-lesional area and of the healthy palms ($p<0.05$). After pain stimulation, sweat secretion was increased significantly in the non-lesional area and the other healthy palm (b) but not in the lesional area.

Figure 3

The PPP lesion begins at the site of the acrosyringium

The PPP lesion shows various histopathological features (a-e). H.E. staining, original magnifications: (a, b, c, e) 200x, (d) 40x. Bar = 50μm

Figure 4

The cells lining the vesicles express the same protein expression as the acrosyringium

Immunostaining of horizontal sections using both GCDFP-15 (a-c) and EMA (d, e) shows expression of both proteins in the normal eccrine ducts (acrosyringium) that looks like pores in the epidermis (positive control) and the lining cells (arrows) of the vesicles. Preimmune IgG was used instead of the dermcidin or hCAP-18/LL37 antibodies as a negative control (f). Original magnifications: (a, d, e) 100x, (b) 40x, (c) 400x. Bar = 50μm

Figure 5

Neither Dermcidin nor hCAP-18/LL-37 is over-expressed in the keratinocytes around the vesicles

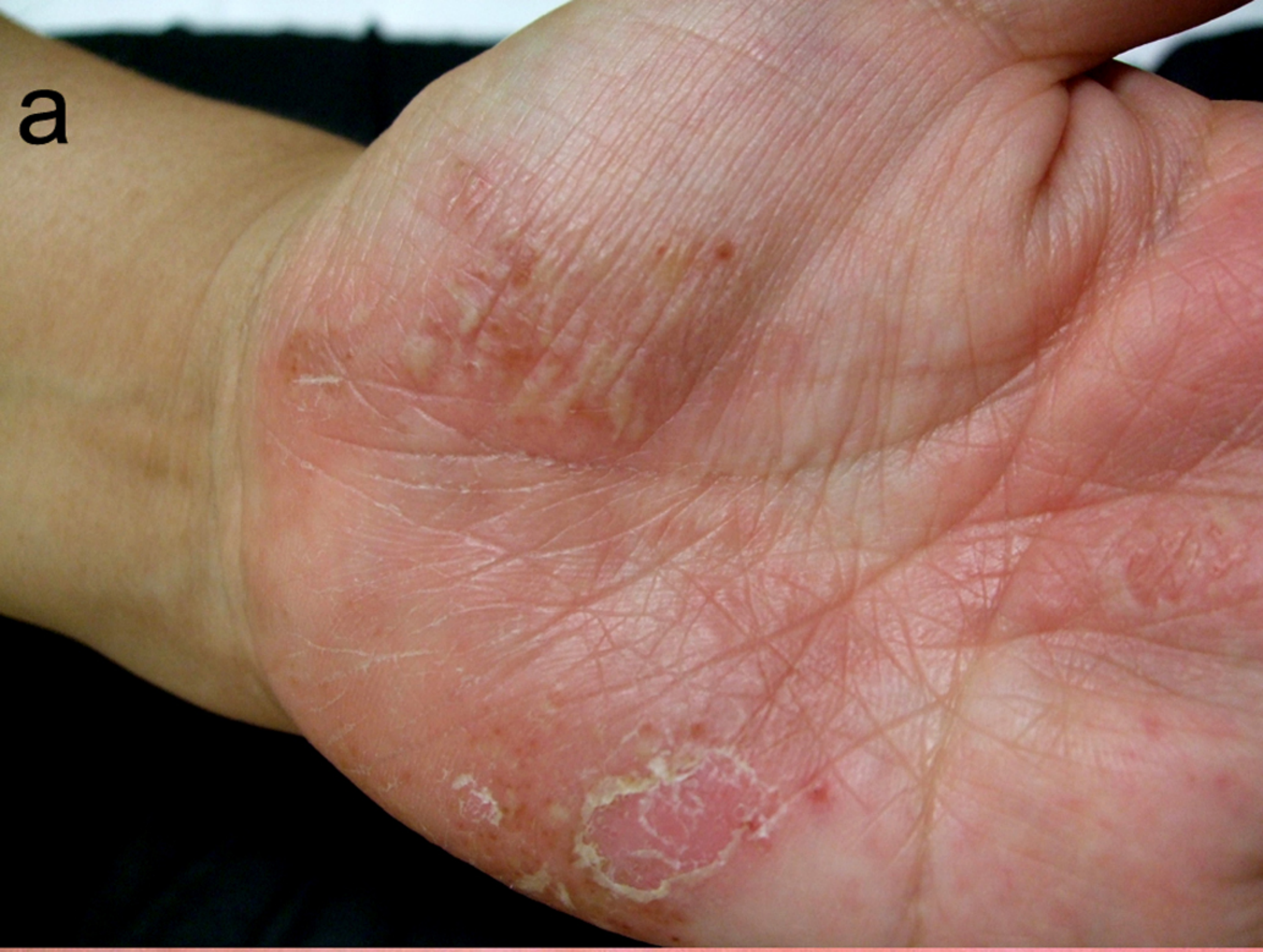
Both dermcidin (a, b) and hCAP-18/LL-37 (c, d) were detected in the sweat duct in the stratum corneum in addition to the eccrine sweat ducts in the dermis (arrows). However, keratinocytes around the vesicles show no dermcidin or hCAP-18 over-expressions (b, d). Preimmune IgG was used instead of the dermcidin or hCAP-19/LL37 antibody as a negative control (e). Original magnifications: 100x, Bar = 50μm

Figure 6

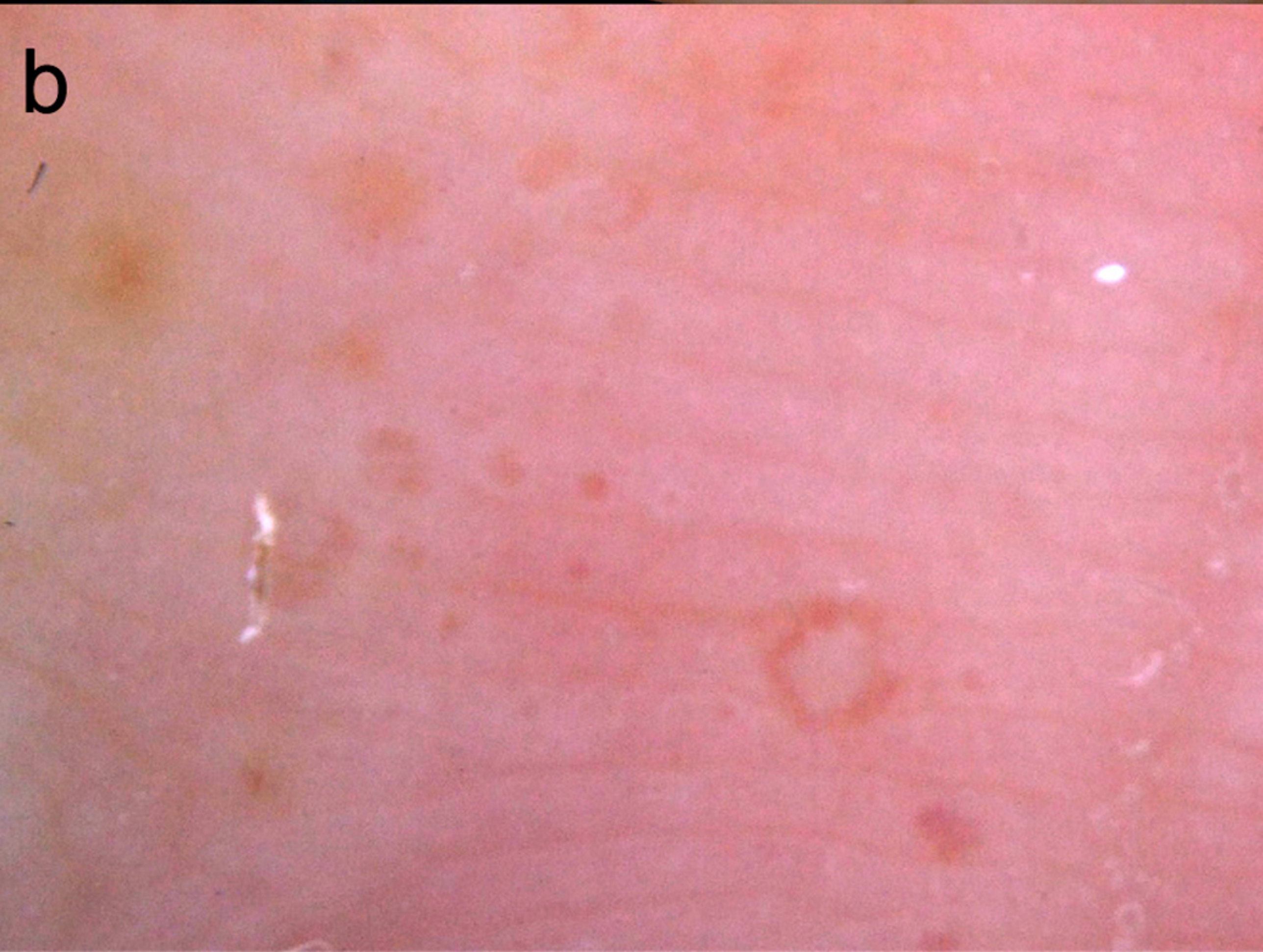
PPP vesicles/pustules contain both dermcidin and hCAP-18/LL-37, however no up-regulation of LL-37 mRNA in lesional PMNs of PPP was seen

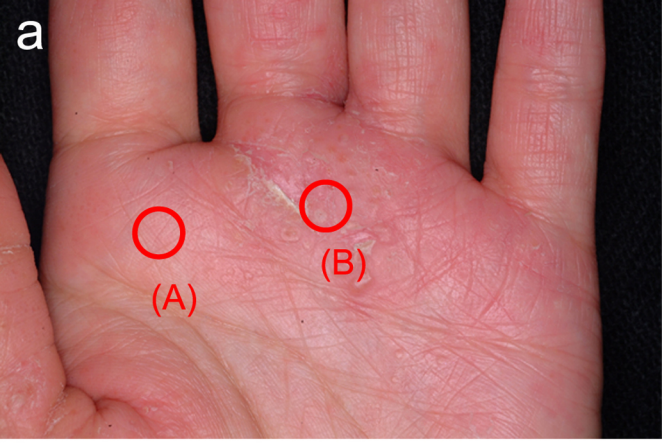
A western blot for hCAP-18/LL-37 shows three bands (18, 15, 4.5 Kd), suggesting the sweat hCAP-18/LL-37 expression pattern as previously described (a). Polymorphonuclear leukocytes of the peripheral blood (PMN) and LL-37 synthetic peptide (LL-37) were applied as positive controls, and dermcidin as a negative control, respectively. The dot-blot showed that vesicles from PPP contain dermcidin (b). Dermcidin synthetic peptide (derm) was applied as a positive control, and dermcidin precursor protein Y-P 30 (Y-P 30) and PMN were applied as negative controls. LL-37 mRNA expression in L- and B-PMNs were analyzed with RT-PCR (c) and real-time PCR (d). RT-PCR showed that both L- and B-PMNs contain hCAP-18/LL-37 mRNA(c). Real-time PCR showed no statistically significant difference in the mRNA expression levels between L- and B-PMNs (d)

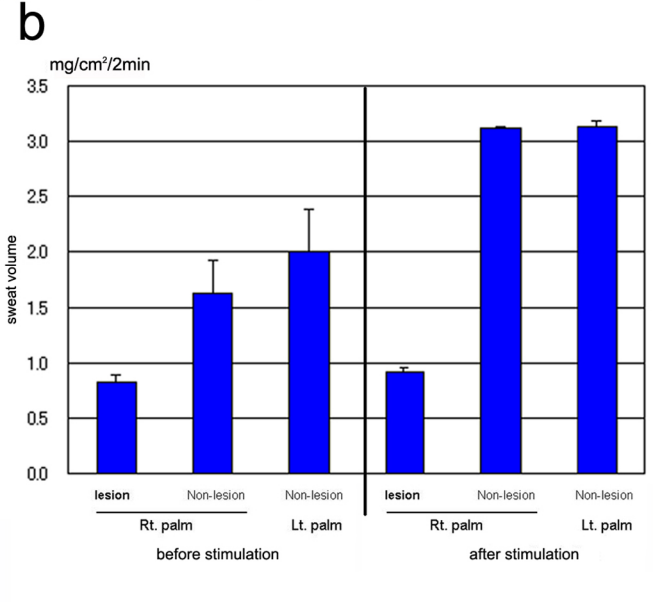
a

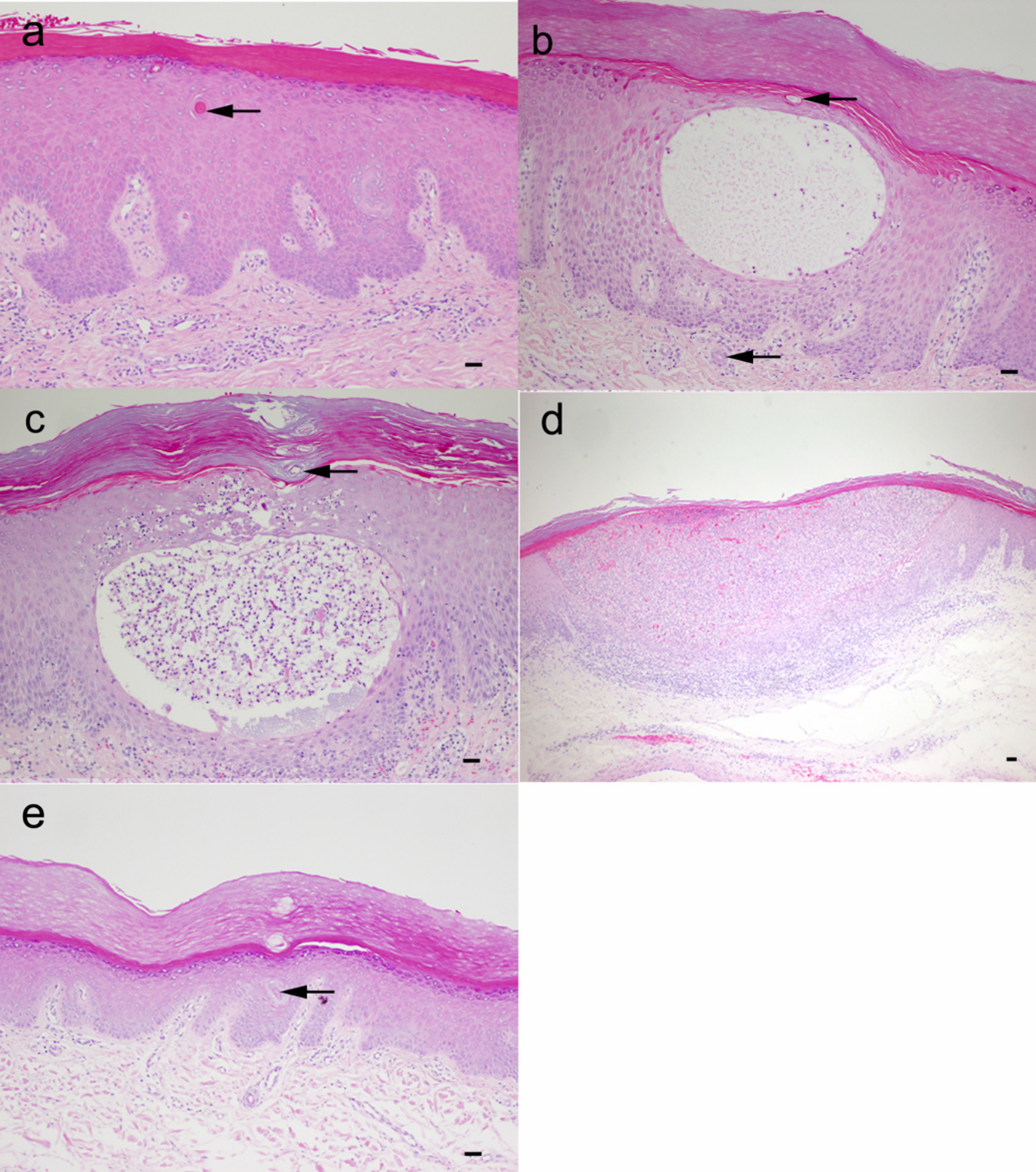


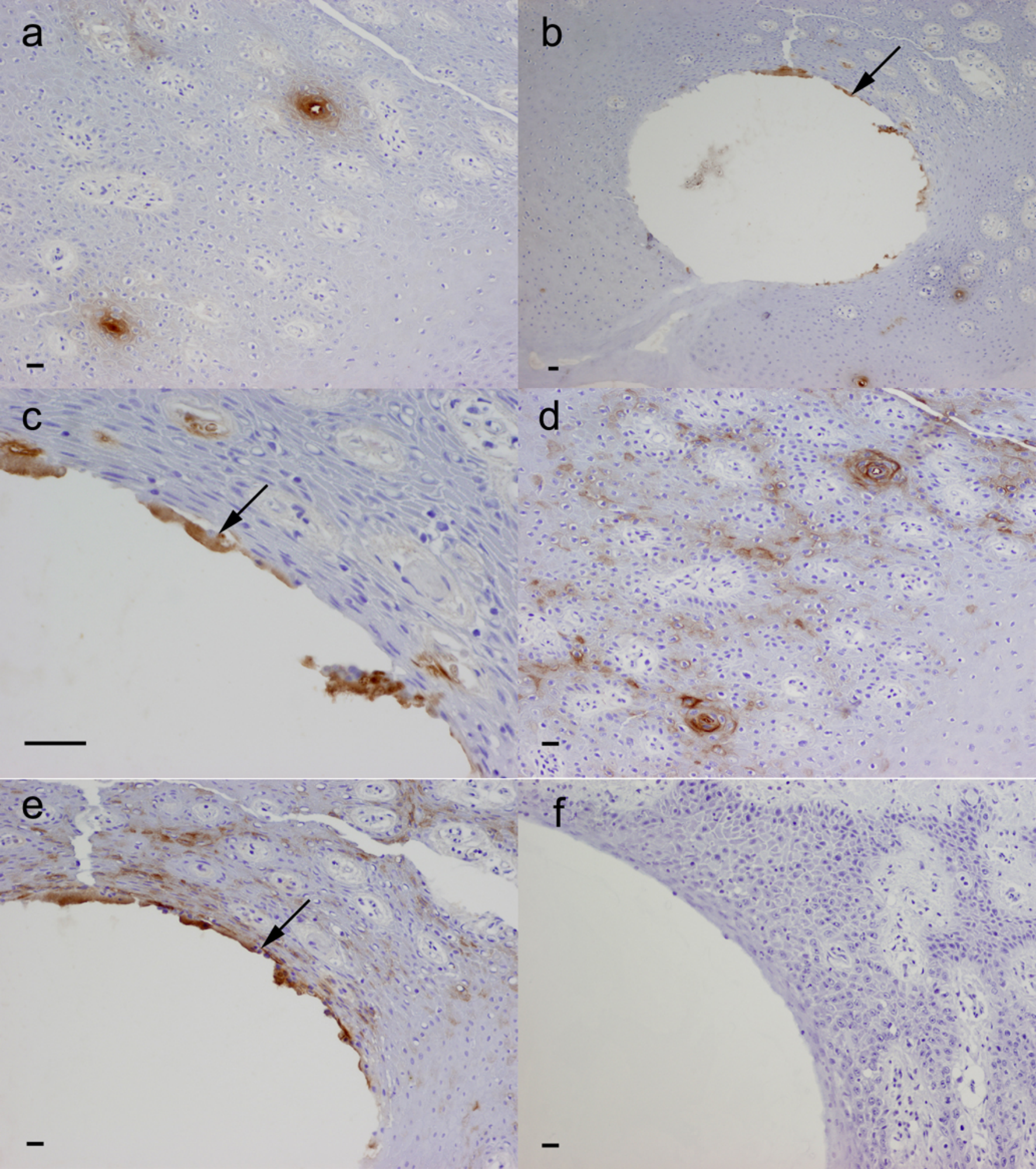
b

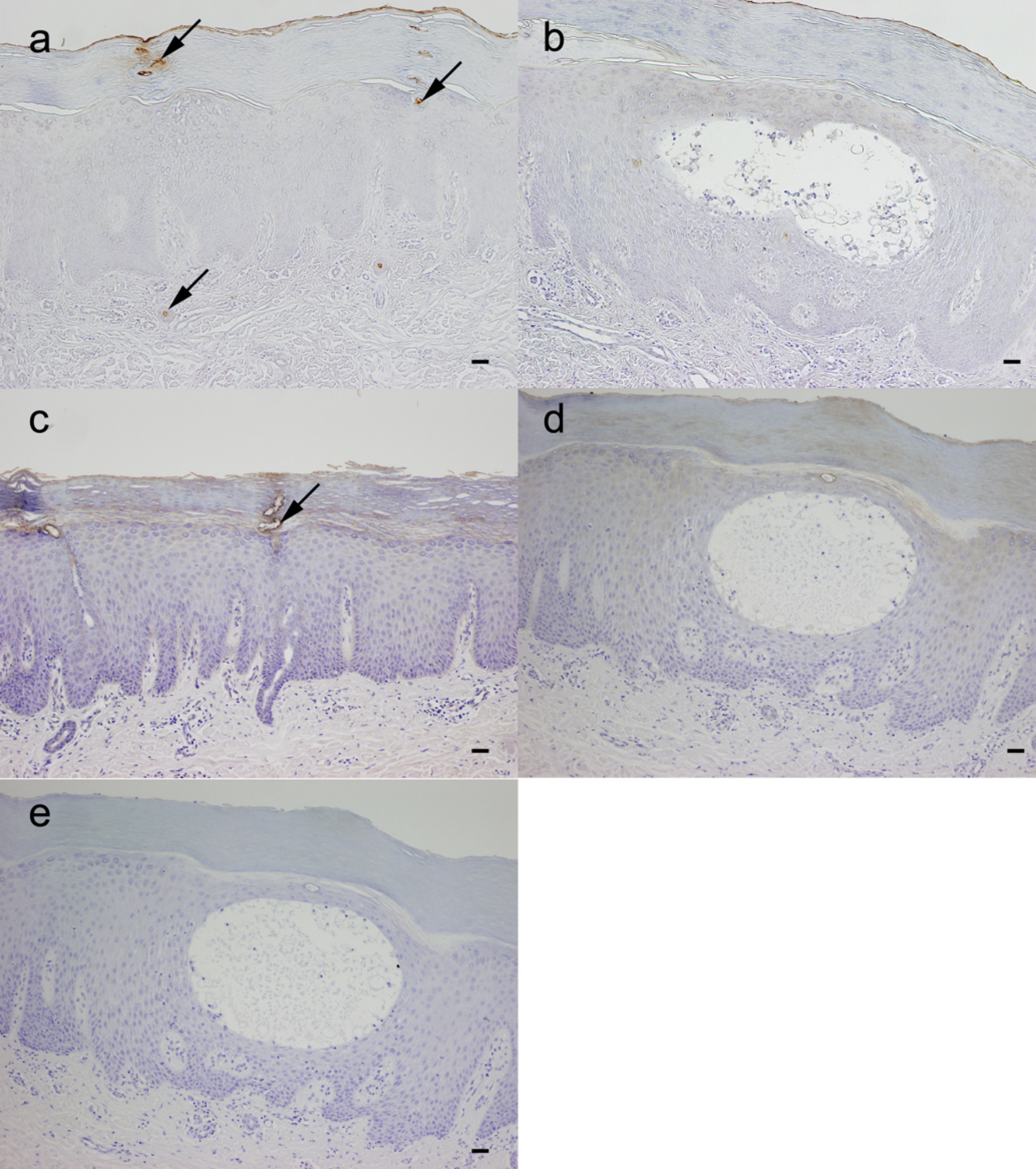


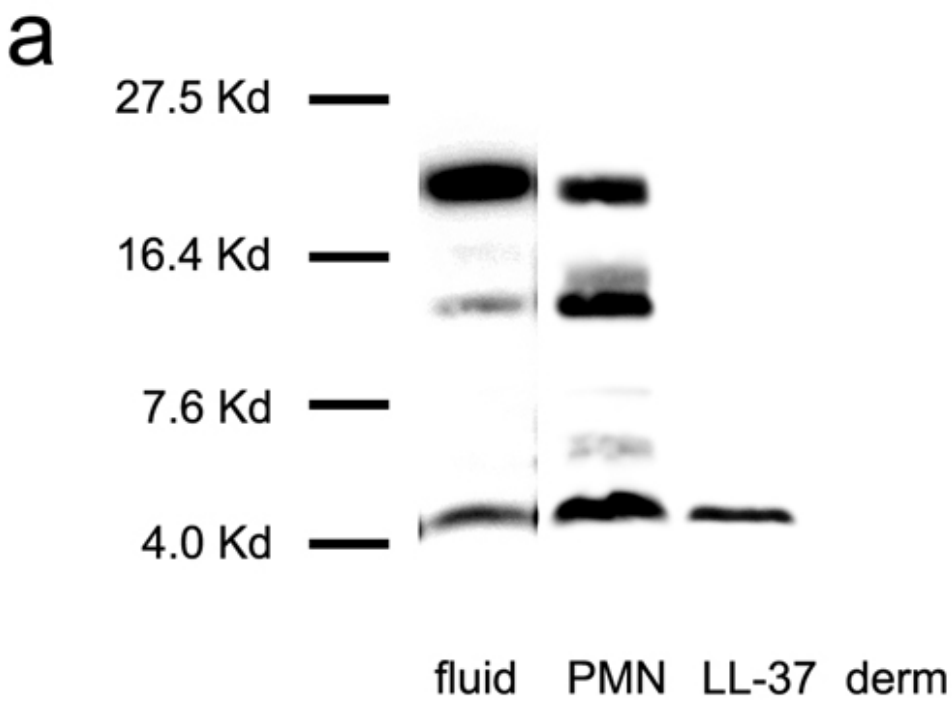

a
(A)
(B)

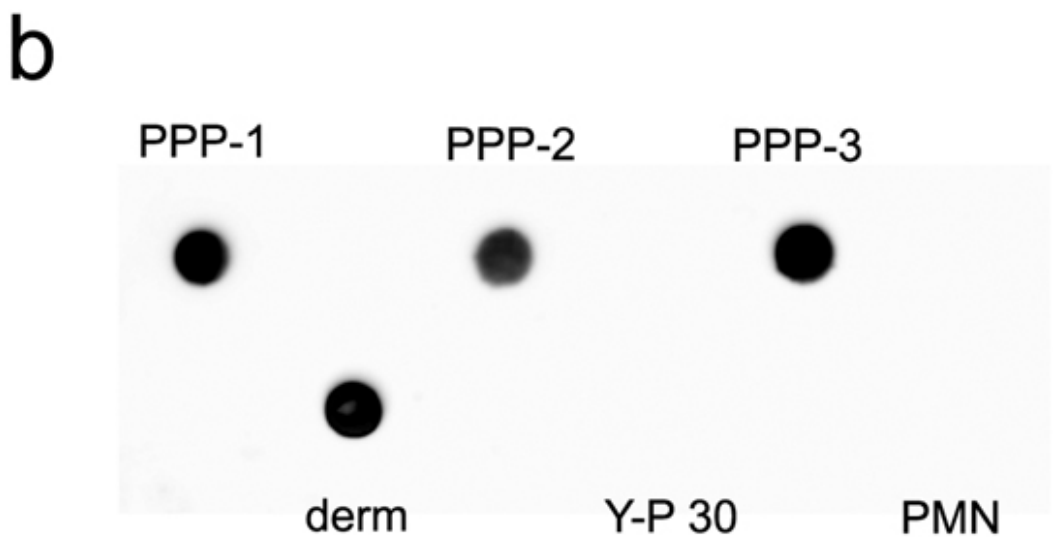

b
mg/cm²/2min
sweat volume
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
lesion
Non-lesion
Non-lesion
Rt. palm
Lt. palm
before stimulation
lesion
Non-lesion
Non-lesion
Rt. palm
Lt. palm
after stimulation

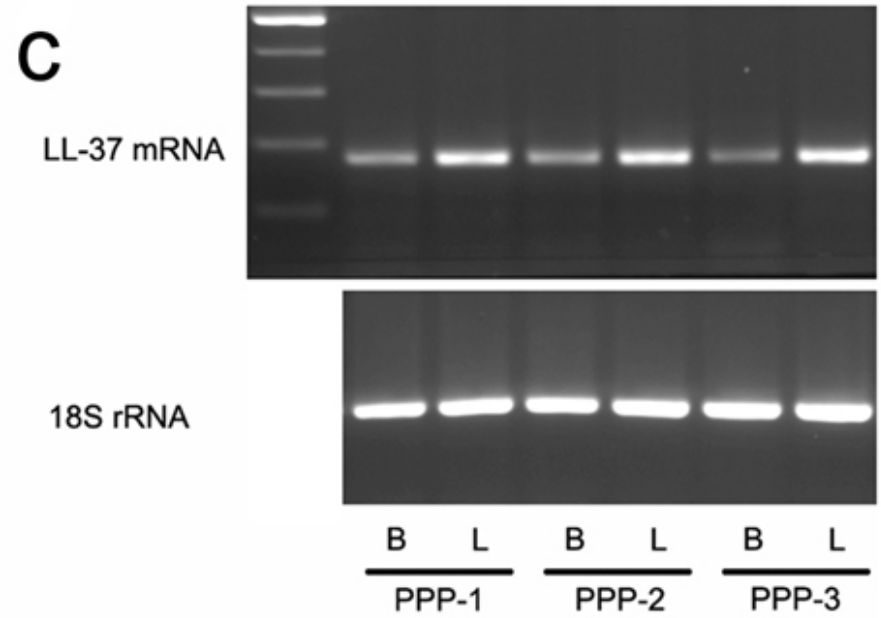

a
b
c
d
e

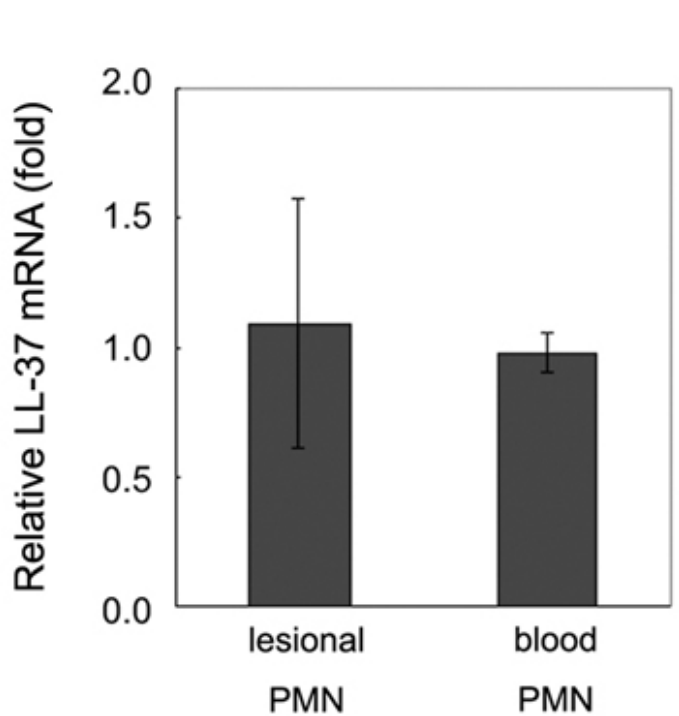

a
b
c
d
e
f


a
b
c
d
e


a
27.5 Kd
16.4 Kd
7.6 Kd
4.0 Kd
fluid
PMN
LL-37
derm


b
PPP-1
PPP-2
PPP-3
derm
Y-P 30
PMN


c
LL-37 mRNA
18S rRNA
B
L
B
L
B
L
PPP-1
PPP-2
PPP-3


d
Relative LL-37 mRNA (fold)
2.0
1.5
1.0
0.5
0.0
lesional PMN
blood PMN