Archives of Dermatological Research (2019) 311:71–82
https://doi.org/10.1007/s00403-018-1882-0

ORIGINAL PAPER

Leakage of sweat into the dermo-epidermal junction as a possible trigger for lichen planus lesion development

Yoshiko Mizukawa[1] · **Yoshimi Yamazaki**[1] · **Tetsuo Shiohara**[1]

Received: 19 February 2018 / Revised: 8 November 2018 / Accepted: 26 November 2018 / Published online: 3 December 2018

Abstract

No previous studies have convincingly linked sweating disturbance with the subsequent development of lichen planus (LP). Therefore, we investigated whether sweating disturbance could be specifically detected in LP lesions and how it could trigger lesion development. We utilized the impression mold technique (IMT), which allows accurate quantification of individual sweat glands/ducts actively delivering sweat in a well-defined location, to evaluate sweating disturbance in LP lesions. Psoriasis vulgaris (PsV) lesions were included as controls. Leakage of sweat and subsequent induction of chemokine expression were immunohistochemically identified. Both baseline and thermal stimulus-induced sweating responses were markedly impaired in LP lesions, as well as in PsV lesions. A marked difference, however, was found in normal-appearing perilesional skin; "cold spots", which were defined as a 1 mm^2 area with no sweat droplets, were specifically and abundantly detected in perilesional LP skin, but not perilesional PsV skin. Leakage of sweat as evidenced by the immunohistochemical detection of dermcidin was specifically observed around the acrosyringium of these "cold spots" in LP, but not PsV, lesions and associated with CXCL10 induction on neighboring keratinocytes and syringotropic migration of $CXCR3^+$ T cells. Leakage of sweat into the dermo-epidermal junction would serve not only to decrease sweat delivery to the skin surface but also to induce T-cell recruitment to the inflammatory site. Therapies for LP may be directed not only at ameliorating inflammatory responses but also at preventing the leakage of sweat into the dermo-epidermal junction.

Keywords CXCL10 · CXCR3 · Dermcidin · Lichen planus · Sweating disturbance

Abbreviations

DCD	Dermcidin
HC	Healthy control
IL	Interleukin
IMT	Impression mold technique
LP	Lichen planus
PsV	Psoriasis vulgaris
TNF	Tumor necrosis factor

Introduction

The majority of clinical and experimental work on lichen planus (LP) has focused on T cells [12, 18, 27, 28, 33–35] while ignoring the identification and characterization of an upstream signal that facilitates the highly directed migration of pathogenic T cells to inflammatory lesions. Thus, it remains unknown how pathogenic T cells are attracted to specific tissue. Earlier studies by Akosa et al. [1, 2], which demonstrated sweat gland abnormalities in LP and other lichenoid dermatoses, provide an important clue to the identification of the upstream signal, although these studies have received little attention.

Eccrine sweat glands have been shown to produce dermcidin (DCD), a novel antimicrobial peptide that has antimicrobial activity against a wide range of pathogens, such as *Escherichia coli, Staphylococcus aureus* and *Candida albicans* [15, 20]. In addition, interleukin (IL)-1α, IL-1β, IL-6, IL-8, IL-31 and tumor necrosis factor (TNF)-α, many of which have been implicated in the induction and maintenance of T-cell migration into the skin at diverse phases of inflammatory responses, have been identified in human sweat [4, 5, 7, 10, 22]. These observations suggest that leakage of sweat into the epidermis and dermis may induce T-cell accumulation and epidermal damage, both of which are typically seen in LP lesions. Although this possibility has

✉ Yoshiko Mizukawa
ymizu@ks.kyorin-u.ac.jp

[1] Department of Dermatology, Kyorin University School of Medicine, 6-20-2, Shinkawa Mitaka, Tokyo 181-8611, Japan

not yet been explored in LP, we have recently demonstrated that leakage of sweat leading to sweating disturbance is specifically detected in lichen amyloidosus (LA) within the spectrum of lichenoid dermatoses, and that the resolution of LA lesions can be induced by restoring sweating disturbance upon treatment with a moisturizer [25].

In this report, we quantitatively evaluated sweating responses at baseline and those following thermal stimulus in involved and perilesional uninvolved areas in LP and psoriasis vulgaris (PsV) patients together with control subjects. We employed an impression mold technique (IMT) that allows accurate quantification of each sweat gland/duct activity in a well-defined location. Our results indicate that each inflammatory disease displays its own unique pattern of sweating disturbance in the lesion. In particular, leakage of sweat into the dermo-epidermal junction was specifically observed in normal-appearing perilesional LP skin upon thermal stimulus and associated with the induction of inflammatory chemokines. Thus, leakage of sweat is likely to represent the earliest event in the immunoinflammatory cascade leading to the recruitment and activation of T cells in LP, but not PsV, lesions, through the induction of inflammatory chemokines.

Methods

Patients and samples

Ten patients with LP (male:female ratio, 7:3; mean age ± SEM, 45.4 ± 6.4 years) and four patients with PsV (male:female ratio, 3:1; mean age ± SEM, 54.0 ± 7.4 years) diagnosed according to their clinical features and histopathological findings were enrolled in this study. Eight healthy volunteers (male:female ratio, 6:2; mean age ± SEM, 31.1 ± 3.6 years) were also enrolled as the healthy control (HC). The clinical data of all biopsy specimens are summarized in Table 1. All subjects gave informed consent and the Institutional Review Board of Kyorin University School of Medicine approved this study (H21-008-03). There was no significant difference in the mean age between LP and PsV patients, and HC. Skin biopsy specimens were obtained from the LP or PsV lesion itself and adjacent perilesional skin. Some samples were obtained 30 min after thermal stimulus for the detection of leakage of sweat into the dermo-epidermal junction.

Table 1 Characteristics of patients in this study

Patients no./ age, years/sex	Involved sites	Sampling of impression mold	Underlying diseases
Lichen planus			
26/F	Leg, buttock	Hip, thigh	
76/M	Leg, foot	Lower leg, foot	
39/M	Buttock, thighs, inguinal region	Thigh	
55/M	Buttock, thighs	Thigh	Hepatitis C
37/M	Extremities	Lower leg, foot	
14/M	Trunk, legs	Leg	
71/F	Leg, foot	Leg	Hypertension, cerebral infarction
27/M	Leg, foot	Leg	Epilepsy
53/F	Buttock	Chest	
56/M	Buttock, extremities	Abdomen, thigh	Hypertension
Psoriasis vulgaris			
35/F	Trunk, extremities	Leg, arm	
54/M	Trunk, extremities	Thigh	Hypertension, hypergricemia
71/M	Thigh, arm	Thigh	
56/M	Trunk, extremities	Leg, arm	
Healthy controls			
26/M		Thigh	
33/M		Arm	
29/F		Thigh	
30/M		Trunk	
24/M		Arm	
26/M		Arm	
55/F		Thigh	
26/M		Arm	

IMT for evaluating sweating disturbance in LP, PsV or HC before and after thermal stimulus

Sweating was induced by immersing both legs or a leg to knee level for 30 min in a water bath maintained at 43 °C, as previously described [26]. All measurements were performed in an air-conditioned room (room temperature, 23 ± 1 °C; and relative humidity, 45 ± 5%) and all patients were allowed to acclimate to this temperature for at least 30 min prior to testing. Heart rate and blood pressure were monitored during thermal stimulus.

To evaluate baseline or thermal stimulus-induced sweating responses, we employed IMT, as described in previous studies including our own [11, 25, 30, 31]. The IMT allows accurate quantification of sweat glands actively delivering sweat and volume of sweat produced. Silicone material was spread onto sweating skin before and after thermal stimulus. As the silicone hardened, it retained the impressions of the sweat droplets as they emerged from the sweat ducts and pushed up into the mold. Impression molds generally hardened within 3–4 min and were subsequently removed. The number and diameter of sweat droplets were counted and measured using a dissecting microscope. IMT samples were obtained from LP and PsV lesions themselves together with clinically normal-appearing perilesional skin before and after thermal stimulus. In some experiment (Fig. 1), IMT samples were also obtained from uninvolved skin on the opposite side as controls. The number of sweat droplets was expressed as that per square centimeter.

To investigate whether sweating disturbance could be detected in the earliest LP lesion, we also determined the number of sweat droplets in normal-appearing perilesional skin, which was further divided into two parts according to the distance from the lesion: 10 mm ≤ and > 10 mm. In addition, to detect the initial sweating disturbance, we examined whether there was a "cold spot" of sweat droplets in perilesional skin. To do this, we calculated the number of areas in which sweating droplets were never detected within the range of 1 mm^2 in perilesional skin, and these areas were defined as "cold spots". For each sample, at least three randomly selected fields were assessed. Samples were analyzed by two independent investigators (YM and YY) in a blinded manner. The assessments of each observer were not significantly different.

Immunohistochemical analysis

Immunohistochemical examinations were performed using serial paraffin sections of formalin-fixed materials, as previously described [25, 30]. Biopsy specimens from patients were obtained in each case, including those from the lesion itself and perilesional uninvolved areas, some of which were obtained before and 30 min after thermal stimulus, as indicated. Control specimens were also collected from patients without any evidence of lichenoid tissue reaction and sweating disturbance, most of which were obtained from benign cutaneous tumors. Biopsy specimens were fixed in 10% buffered formaldehyde and embedded in paraffin. Sections were stained with hematoxylin and eosin for routine histological examination. Other sections were deparaffinized and rehydrated. Then, sections were stained using a two-step immunostaining kit (DAKO EnVision™ + Dual Link, No. 4063; DAKO, Glostrup, Denmark). In brief, biopsy sections were incubated with primary antibodies overnight at 4 °C. The following primary antibodies were used: anti-DCD mAb (sc-33656; Santa Cruz, CA, USA), anti-CXCL10 mAb (500-P93, Peprotech, NJ, USA) and anti-CXCR3 mAb (557183, BD Pharmingen, NJ, USA). Specimens were then incubated with horseradish peroxidase-conjugated goat anti-mouse IgG for 30 min at room temperature. In some experiments, double staining for CXCL-10 and CD3 was performed using anti-CXCL-10 mAb followed by anti-CD3 mAb (A0452, DAKO). Enzyme reactions were developed with conventional substrates for chromogen (K3464, DAKO).

Statistical analysis

Data are expressed as mean ± SEM and significant differences between groups were determined using one-way ANOVA, Tukey–Kramer's test or Dunnett's test. Significance was defined as p value of 0.05 or less for all tests.

Results

Evidence suggestive of sweating disturbance in the development of LP

The starch–iodine test performed on LP lesions (Fig. 1a, b) under baseline conditions without any stimulus showed reduced baseline sweating at the affected site. In IMT measurement, baseline levels of sweating responses without any stimulus, as evidenced by the number of sweat droplets, were markedly reduced in lesional skin, while no significant sweating disturbance was observed in uninvolved skin on the opposite side compared with those in HC (Fig. 1c). These results prompted us to investigate whether sweating disturbance, as evidenced by a decrease in the number of sweat droplets, could also be detected even after thermal stimulus in LP lesional and perilesional skin. In addition, we also evaluated how sweating disturbance could trigger lesion development.

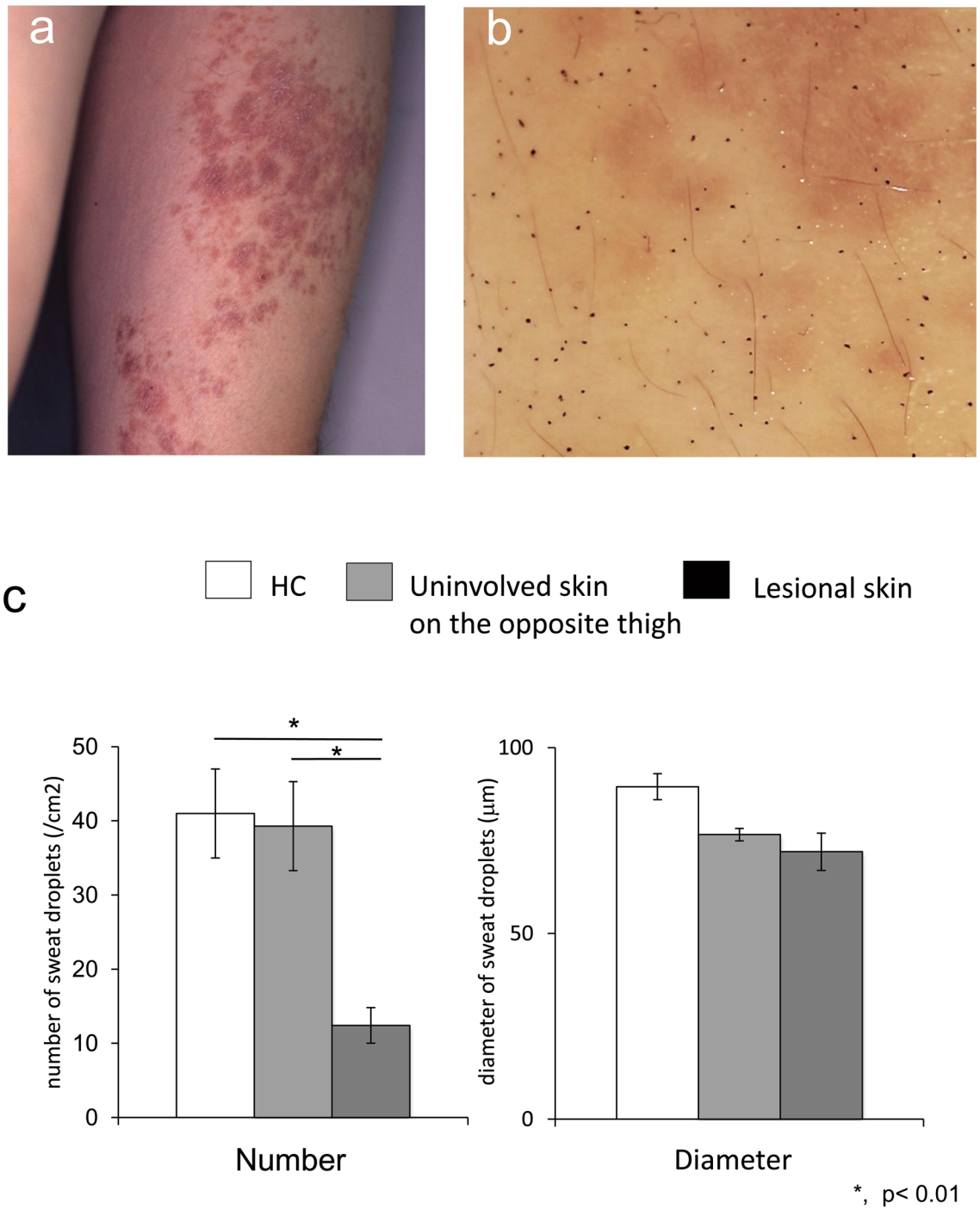

Fig. 1 Starch–iodine test and IMT under baseline conditions without any stimulus in lesional LP skin, uninvolved normal-appearing skin on the opposite thigh of the LP patient or HC. **a** Clinical findings of representative LP lesions. Multiple violaceous, erythematous papules are noted in the inner aspect of the thigh. **b** The starch–iodine test showed a reduced sweating response limited to lesional and perilesional LP skin. **c** In the IMT, the number of sweat droplets in the thigh from the HC (white bar) and uninvolved skin on the opposite thigh of the LP patient (gray bar) and LP lesional (dark bar) skin in the thigh under baseline condition. Data are expressed as the mean ± SEM ($n = 6$). *$p < 0.05$

Sweating responses to thermal stimulus in control subjects, and LP and PsV patients

The number and size of sweat droplets in HC peaked at 30 min and the sweat droplets thus detected were evenly distributed in the area tested and typically located at the folds (Fig. 2). As shown in Figs. 3 and 4, the number and diameter of sweat droplets detected in LP and PsV lesions 30 min after thermal stimulus were profoundly decreased compared with those in HC (Fig. 2). Even at the perilesional

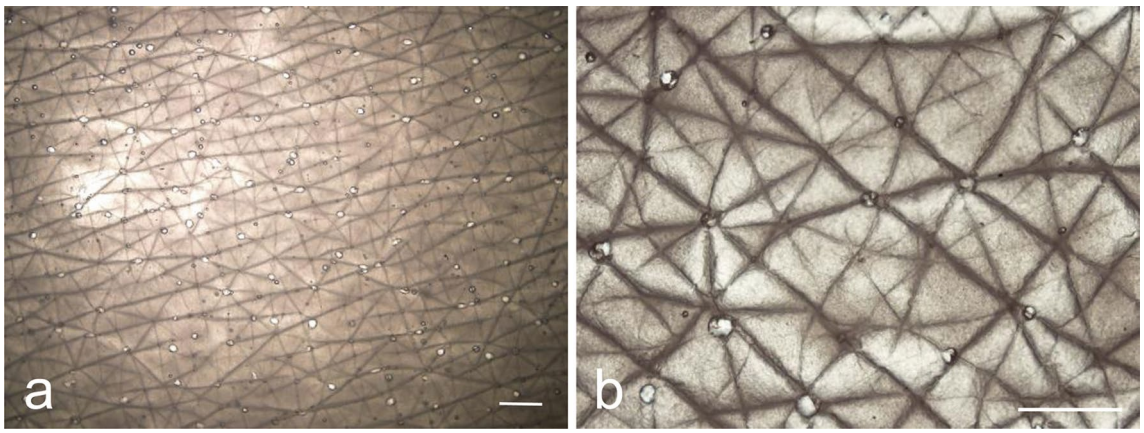

Fig. 2 Representative silastic molds obtained from the thigh of HC 30 min after thermal stimulus. **a** Sweat droplets are evenly distributed. Bar 1 mm. **b** Close-up view of the sweat droplets usually detected at the folds. Bar 500 µm

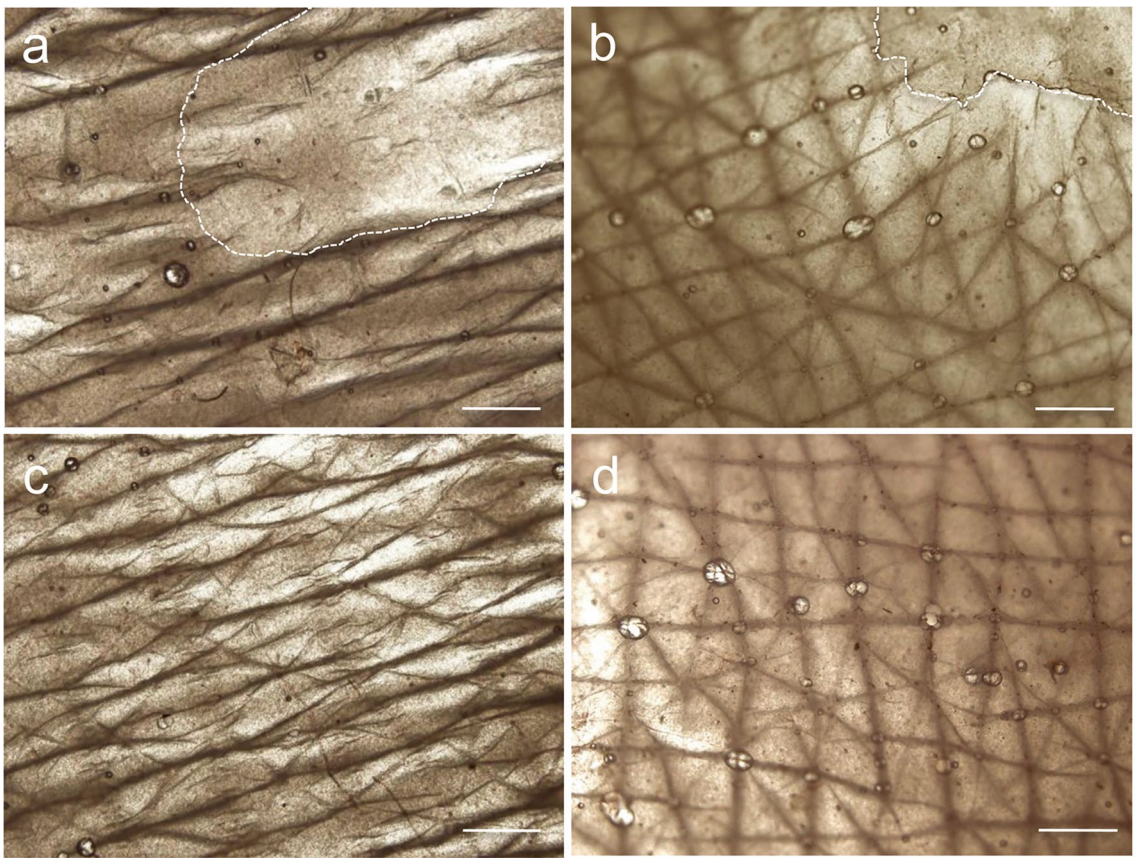

Fig. 3 Representative silastic molds from LP (left) or PsV (right) lesions 30 min after thermal stimulus. High-power field view of LP (**a**) and PsV (**b**) lesional and perilesional areas in the thigh. High-power field view of perilesional skin (< 10 mm away from lesions) of LP (**c**) and PsV (**d**). Bars 500 µm. The lesional area is indicated by dotted lines. Sweat droplets are visualized as small holes corresponding to sweat pores

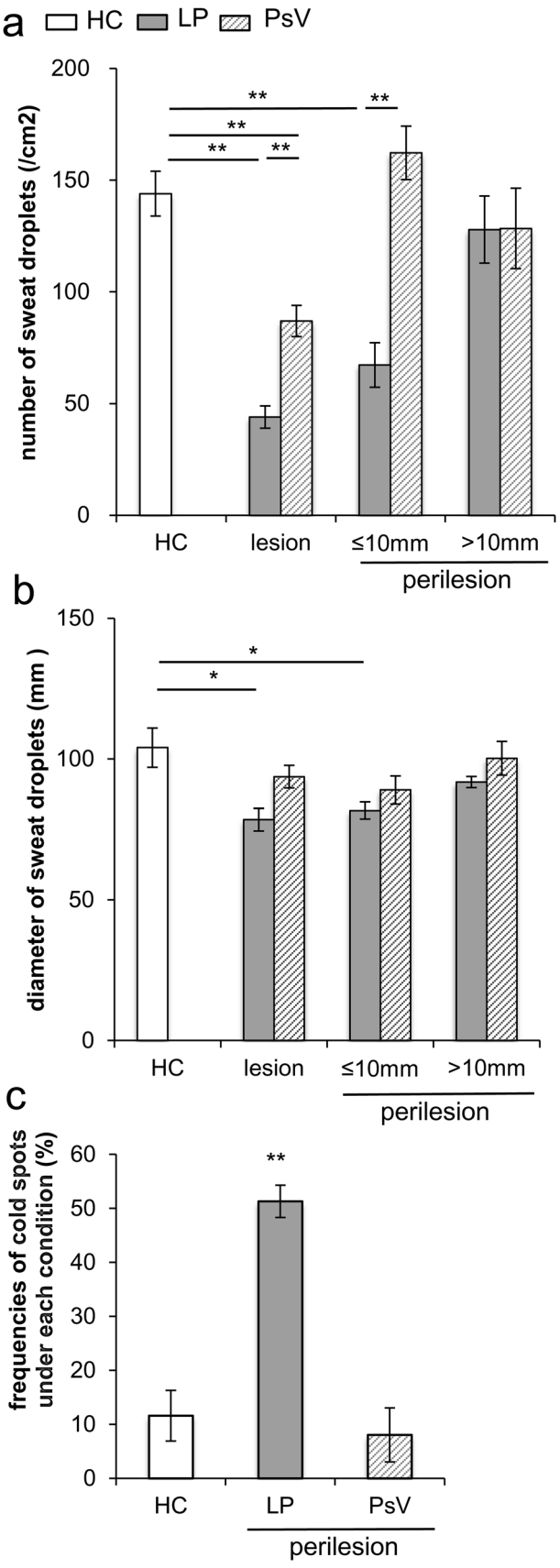

Fig. 4 Differences in the mean number and diameter of sweat droplets 30 min after thermal stimulus in LP or PsV lesions. **a** The mean number of sweat droplets in HC (white bar) and in the perilesional skin site 10 mm ≤ and > 10 mm away from LP (gray bar) and PsV (hatched bar) lesions. **b** The mean diameter of sweat droplets in each condition. **c** Frequencies of "cold spots" in normal-appearing perilesional skin of these lesions. Frequencies of "cold spots" were calculated using the following formula: [the number of cold spots without sweat droplets in the perilesional skin / the number of cold spots without sweat droplets + the number of non-cold spots with any sweat droplets in the perilesional skin] × 100. For each sample, at least three randomly selected fields were assessed. Data are expressed as the mean ± SEM. $*p < 0.05$, $**p < 0.01$

site, a profound decrease in the number and diameter of sweat droplets was observed in LP (Figs. 3a, c, 4a), but not PsV (Figs. 3b, d, 4a), lesions. Thus, sweat volume per square centimeter was profoundly decreased in lesional and perilesional LP skin (data not shown). There was no significant regional difference in the extent of the decrease in the numbers of sweat droplets in LP lesions according to the regions examined, as far as we examined the lower legs, thigh and abdomen.

More importantly, a marked, pin-point loss of sweat droplets, which we referred to as "cold spot", was also observed in the uninvolved perilesional site of LP (Fig. 3c). This droplet-loss area was clinically invisible at the time of IMT but clinically manifested within 2–3 days unless treated (data not shown), suggesting that this area would be destined to become lesional. The preferential abundance of cold spots at the perilesional site was confirmed in the skin site < 10 mm away from LP lesions (Fig. 3c), but not the corresponding skin site in PsV lesions (Fig. 3d). We next investigated how many "cold spots", which were defined as a 1-mm^2 area with no sweat droplets, could be detected in normal-appearing perilesional skin (< 10 mm away from the lesion) in LP and PsV. As shown in Fig. 4c, frequencies of "cold spots" were the highest in the perilesional LP skin. In contrast, "cold spots" were rarely found in perilesional PsV skin; in other words, any sweating disturbance could not be detected in the perilesional PsV skin. Thus, the marked difference in sweating disturbance between LP and PsV was typically observed in normal-appearing perilesional (< 10 mm) LP and PsV skin. These results suggest that the abundance of "cold spots" in normal-appearing perilesional skin is a hallmark of LP. This finding suggests the involvement of sweating disturbance as a trigger for the development of LP.

Superficial blockage of sweat ducts responsible for decreased sweating capacity

The abovementioned data could be alternatively interpreted as an absence of active eccrine glands/ducts able to produce sweat in the LP lesion. Thus, to test the ability of sweat glands to produce sweat, we investigated whether

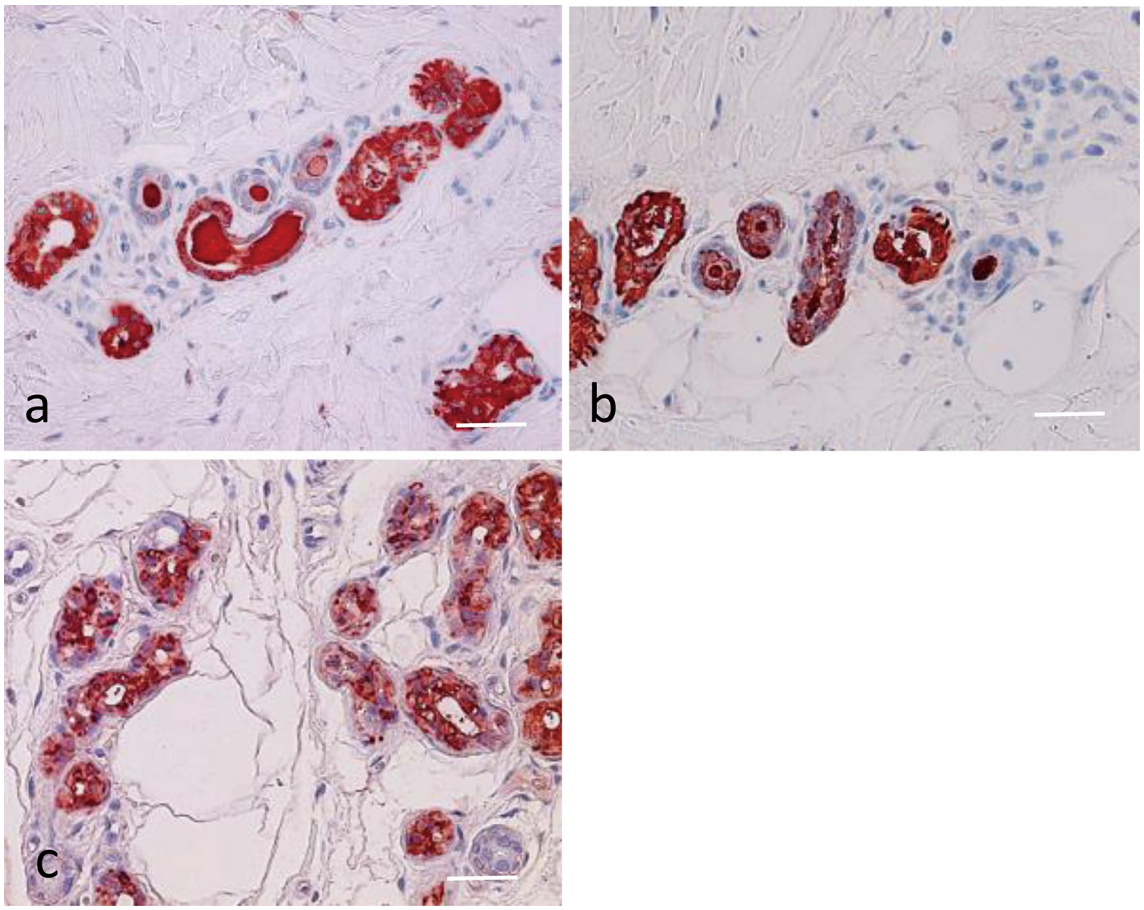

Fig. 5 Immunohistochemical detection of DCD expression in sweat glands in LP (**a**) and PsV (**b**) lesions, and control specimens obtained from benign cutaneous tumors (**c**). Biopsy specimens were obtained under baseline conditions without any stimulus to induce sweating. Bars 20 µm

dermcidin (DCD) specifically produced by sweat glands could be detected in sweat glands in lesional skin [19, 23]. As shown in Fig. 5, DCD was expressed in the cytoplasm of dark cells and luminal membranes of eccrine glands, but not in sweat ducts, in LP (Fig. 5a) and PsV lesions (Fig. 5b), and HC (Fig. 5c). Strong staining for DCD was also detected in amorphous, intraluminal materials seen in the enlarged lumen of glands and ducts in LP, suggesting retention of sweat (Fig. 5a). The amorphous materials suggestive of retention of sweat were detected in approximately > 90% sweat glands/ducts in the LP lesions. After thermal stimulus, however, the amorphous materials were markedly decreased, but remained detected in the lumen of the sweat glands (data not shown). These findings suggest that sweat glands in LP lesions have sufficient ability to produce sweat, but the ability to deliver sweat to the skin surface could be impaired by unknown mechanisms. However, because keratinous plugs capable of causing sweat duct obstruction were never observed in the "cold spots" of the perilesional site of LP lesions, defective sweat delivery to the surface observed in LP lesions could be due to the functional defects or duct fragility.

Leakage of sweat into the dermo-epidermal junction leading to the development of LP lesions could be investigated if it were possible to identify an area of clinically normal skin that was destined to become lesional skin. We, therefore, investigated whether leakage of sweat could be detected after, but not before, thermal stimulus in a cold spot of perilesional LP skin (Fig. 6a). As shown in Fig. 6b, c, cold spots were found in lesional and perilesional LP skin of the dorsal surface of the foot, where skin surface patterns composed of skin folds and ridges were not yet disturbed. As shown in Fig. 6e, f, DCD expression in intraluminal materials, i.e., retention of sweat, was detected up to the portion around the dermo-epidermal junction in normal-appearing perilesional LP skin under baseline condition. Positive staining for DCD was only observed in the lumen of the acrosyringium in the lower basal epidermis (closed arrow in Fig. 6f), suggesting that sweat delivery to the skin surface would be functionally impaired in ducts at levels close to the dermo-epidermal junction.

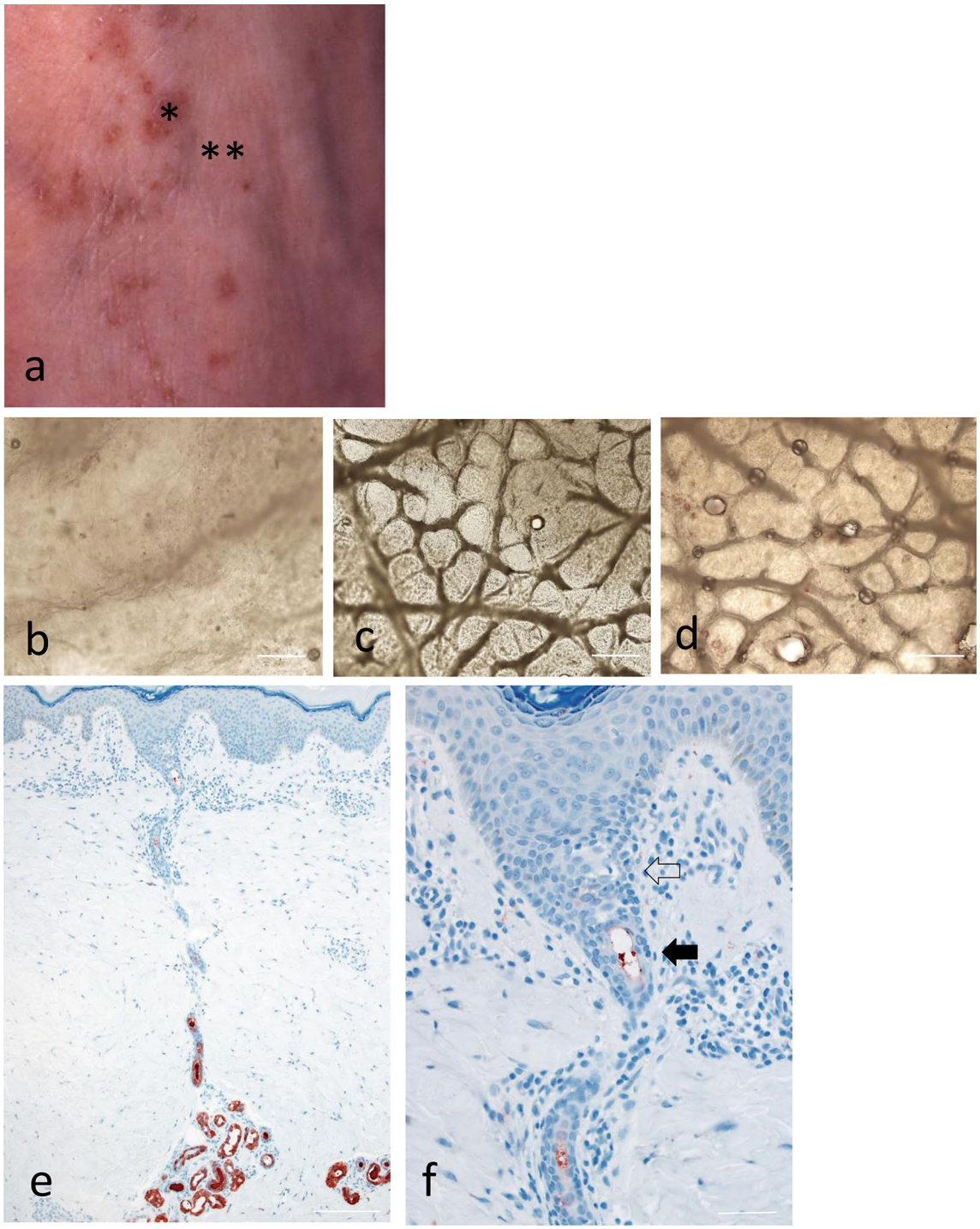

Fig. 6 Relationship among clinical findings, silastic molds and DCD expression in LP lesion and clinically normal-appearing perilesional skin. **a** Clinical finding of LP lesion (asterisk) and perilesional skin (double asterisk) in the dorsal foot of a patient with LP. **b** Silastic molds in lesional skin 30 min after thermal stimulus. Bars 500 µm. **c** Silastic molds in normal-appearing perilesional skin 30 min after thermal stimulus. **d** Silastic molds in uninvolved skin on the opposite side 30 min after thermal stimulus. **e** DCD expression in eccrine glands and ducts in clinically normal-appearing perilesional LP skin under baseline conditions. Bars 100 µm. **f** High-power field view of **e**. DCD expression in the upper part of dermal and intraepidermal eccrine ducts under baseline conditions. Expression of DCD-positive dots is restricted to the lower part of the epidermis (closed arrow), but not the middle part (open arrow), suggesting impairment of sweat delivery at this point. Bars 20 µm

Induction of chemokine receptor by leakage of sweat

We hypothesized that if leakage of sweat into the epidermis was actually an initial event necessary for T-cell recruitment in LP lesions, then inflammatory chemokines, such as CXCL10 able to attract a specific T-cell subset (e.g., $CXCR3^+$ effector memory T cells), would be induced upon leakage of sweat into the dermo-epidermal junction in the cold spots of normal-appearing perilesional skin. Indeed, a previous study clearly demonstrated that DCD can stimulate keratinocytes to express CXCL10 [17].

As shown in Fig. 7a, immunohistochemistry of the cold spots in normal-appearing perilesional LP skin showed that DCD was specifically detected around eccrine ducts within the epidermis (acrosyringium) when biopsy specimens were obtained at 30 min after thermal stimulus. In contrast, no DCD expression was detected around the acrosyringium in PsV lesions even after thermal stimulus (Fig. 7b). Importantly, the presence of DCD around the acrosyringium in perilesional LP skin was associated with CXCL10 expression by the surrounding keratinocytes (Fig. 7c) and syringotropic migration of $CXCR3^+$ T cells (Fig. 7e), a finding not observed in PsV lesions (Fig. 7d, f). Syringotropic infiltrates were composed of $CD3^+$ T cells: $CD3^+$ T cells infiltrated into $CXCL10^+$ epidermal keratinocytes in earlier LP lesions (Fig. 8), at which time epidermal damage was not yet detected. These results indicate that leakage of sweat around the acrosyringium would represent the actual early event that can trigger migration of $CXCR3^+$ T-cell infiltrates directed toward the intraepidermal portion of eccrine ducts through the induction of CXCL10 expression by keratinocytes.

Discussion

In this study, we have demonstrated with the use of IMT that "cold spots", defined as a 1-mm^2 area without sweat droplets, were specifically and abundantly detected in normal-appearing perilesional LP skin, but not normal-appearing perilesional PsV skin. In addition, after thermal stimulus, leakage of sweat around the acrosyringium was immunohistochemically identified in the "cold spots", which became lesional unless treated. Thus, leakage of sweat into the acrosyringium could act as a trigger for the development of LP lesions and result in localized hypohidrosis, as evidenced by the abundance of cold spots in normal-appearing perilesional LP skin. The abundance of cold spots in normal-appearing perilesional LP skin is unlikely to indicate a secondary event that requires the prior lymphocytic infiltrates and rather represents the actual early event that triggers syringotropic lymphocytic infiltrates. In contrast, sweating disturbance in PsV lesions would represent secondary events that require prior inflammation.

How does sweating disturbance trigger syringotropic lymphocytic infiltrates? Here, we clearly demonstrated that sweat pours into the dermo-epidermal junction due, in part, to functional duct blockage or duct fragility at the acrosyringium, thereby causing syringotropic migration of $CXCR3^+$ T cells toward $CLCX10^+$ keratinocytes. In support of this view, recent studies have demonstrated that DCD could stimulate keratinocytes to express a variety of cytokines/chemokines, such as TNF-α, CXCL10 and CXCL8 [17]. A consensus has emerged from these and other studies [13, 32, 33, 35] that CXCR3 and CCR5, which preferentially mark effector/memory T cells, are cell-surface chemokine receptors that guide the T cells to inflammatory sites while CXCR4 and CCR7 are associated with constitutive migration of T cells through lymph nodes [8, 16]. Because CXCR3, expressed primarily on activated effector/memory T cells that infiltrate inflammatory sites, is involved in Th1-biased immune responses [21], activated Th1 effector/memory T cells could efficiently migrate to CXCL10-expressing keratinocytes in LP lesions. Thus, because of inclusion of human sweat, such as proteolytic enzymes or many kinds of cytokines, the proinflammatory recruitment of T cells would be initiated and further amplified by the leakage of sweat in the dermo-epidermal junction. Nevertheless, we could not totally exclude the possibility that leakage of sweat around acrosyringium could represent a secondary event to the epidermal damage induced by lichenoid inflammation. Our data, however, that leakage of sweat around acrosyringium observed in cold spots after thermal stimulus was only associated with non-epidermotropic marginal cellular infiltrates but with subsequent development of LP lesions in some of the cold spots while the lichenoid infiltrates was never associated with leakage of sweat could be interpreted as suggesting that leakage of sweat could be necessary but not sufficient for initiating a complex sequence of events leading to CXCL10 expression on keratinocytes and epidermotropic migration of $CXCR3^+$T cells. Interestingly, similar leakage of sweat into the dermo-epidermal junction was also observed in LA lesions [25]. A difference, however, was noted in the distribution pattern of hypohidrosis areas between LP and LA; in particular, no "cold spots" were detected in perilesional LA skin [25]. This may help to explain the different clinical pictures of both lichenoid dermatosis: LP papules often coalesce into plaques while LA papules do not.

It remains unknown, however, how sweat can migrate into the epidermis in LP lesions despite the presence of tight junctions (TJs). TJs, which are found in epithelial cells have been thought to play a role in maintaining permeability barrier function, thereby preventing free diffusion of macromolecules. The acrosyringium lacks

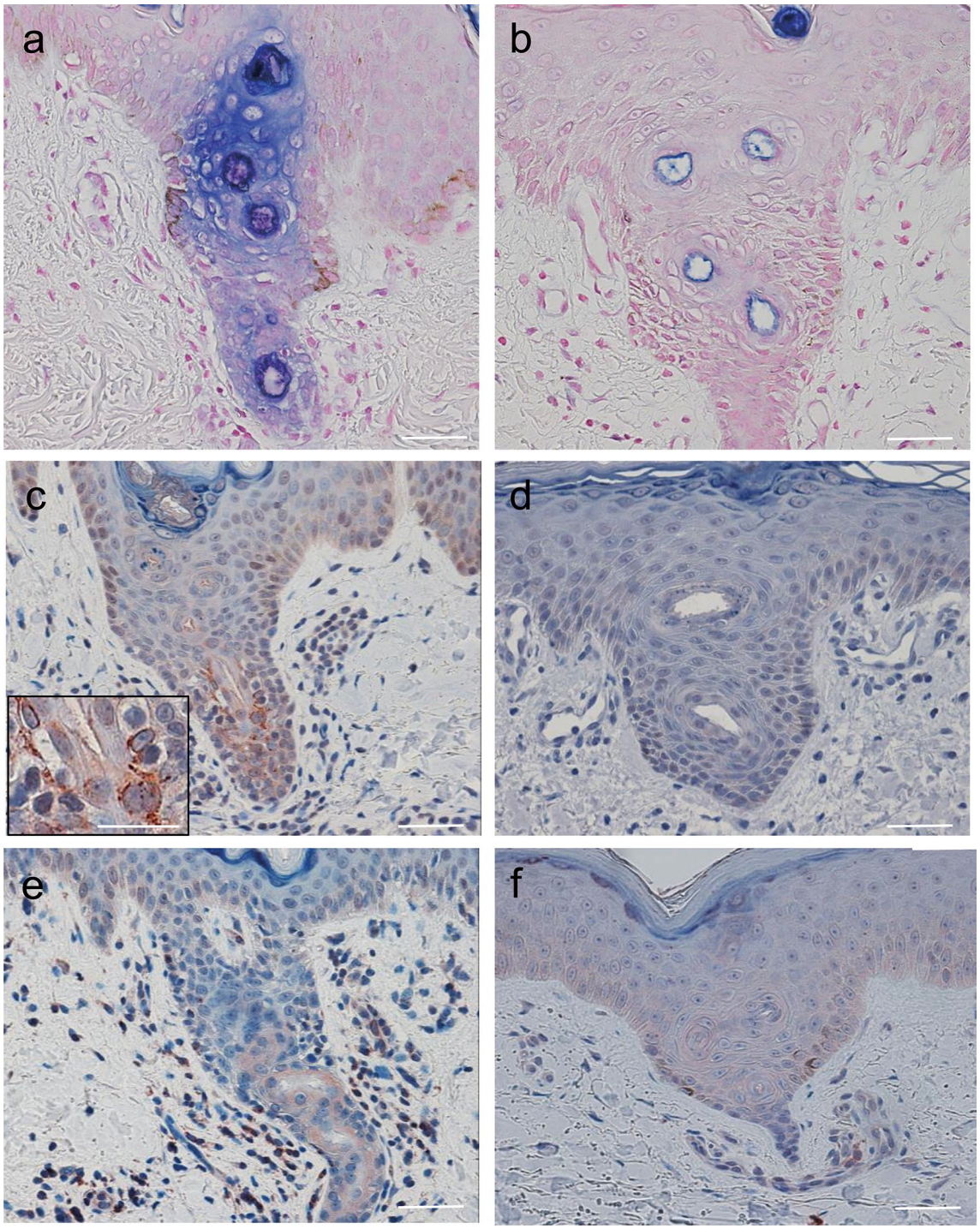

Fig. 7 Expression of DCD, CXCL10 and CXCR3 30 min after thermal stimulus in normal-appearing perilesional skin of LP (left) and PsV (right) lesions. **a** Leakage of DCD into keratinocytes around the acrosyringium in LP lesions. **b** DCD expression was found only in the lumen within the acrosyringium in PsV lesions, but not by keratinocytes. **c** CXCL10 expression by keratinocytes around the acrosyringium in LP lesions. Inset, close-up view of $CXCL10^{+}$ keratinocytes. **d** No CXCL10 expression by keratinocytes around the acrosyringium was observed in PsV lesions. **e** CXCR3 expression by syringotropic T-cell infiltrates beneath the epidermis of LP lesions. **f** No expression of CXCR3 by T-cell infiltrates beneath the epidermis was observed in PsV lesions. Bars 20 µm (**a**–**f**) and 10 µm (inset)

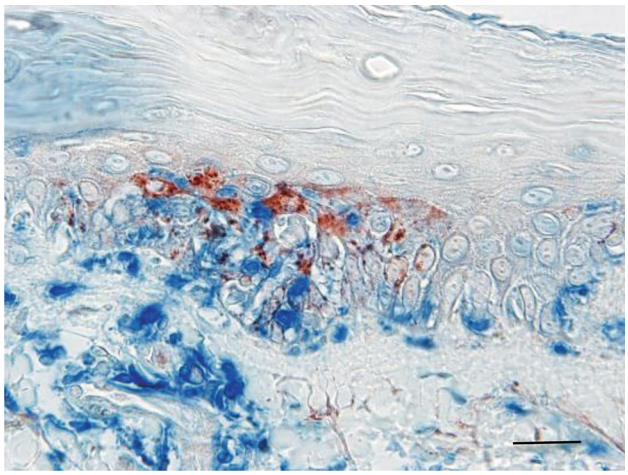

Fig. 8 CXCL10 expression (red) by keratinocytes and CD3 expression (blue) on the infiltrating T cells in the earlier LP lesions. Adherence of $CD3^+$ T cells to $CXCL10^+$ epidermal keratinocytes was observed in the lesions. Bars 20 μm

functional TJ and thus may allow the leakage of sweat. Because leakage of sweat rarely occurs in diverse physiological and pathological settings other than LP and lichenoid dermatoses, the acrosyringium could have an alternative barrier system. In this regard, there have been some reports indicating that herpesvirus infection could alter TJ function [6, 29], suggesting that viral infection may also affect the alternative barrier system. Consistent with this possibility, a number of skin diseases including LP have been shown to develop in the previously involved site of herpes zoster [3, 14, 24].

Our results suggest that the leakage of sweat into the dermo-epidermal junction may be recognized as an early event with specific contribution to disease expression in LP. Many pathological events preceding and during inflammatory responses could be attributed to the effects of sweat migrating into the epidermis. Thus, therapies for LP could be directed not only at ameliorating inflammatory responses in the lesion itself but also at preventing the leakage of sweat into the dermo-epidermal junction in lesional and perilesional skin, as successfully treated in LA [25].

Funding None.

Compliance with ethical standards

Conflict of interest There is no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional (H21-008-03) and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent All subjects gave informed consent and the Institutional Review Board of Kyorin University School of Medicine approved this study (H21-008-03).

References

1. Akosa AB, Lampert IA (1990) The sweat gland in graft versus host disease. J Pathol 161:261–266
2. Akosa AB, Lampert IA (1991) Sweat gland abnormalities in lichenoid dermatosis. Histopathology 19:345–349
3. Braun RP, Barua D, Masouye I (1998) Zosteriform lichen planus after herpes zoster. Dermatology 197:87–88
4. Dai X, Okazaki H, Hanakawa Y, Murakami M, Tohyama M, Shirakata Y, Sayama K (2013) Eccrine sweat contains IL-1α, Il-1β and IL-31 and activates epidermal keratinocytes as a danger signal. PLoS One 8:e67666. https://doi.org/10.1371/journal.pone.0067666
5. Didierjean L, Gruaz D, Frobert Y, Grassi J, Dayer JM, Saurat JH (1990) Biologically active inerleukin 1 in human eccrine sweat: site-dependent variations in alpha/beta ratios and stress-induced increased excretion. Cytokine 2:438–446
6. Dingwell KS, Johnson DC (1998) The herpes simplex virus gE-gI complex facilitates cell-to-cell spread and binds to components of cell junctions. J Virol 72:8933–8942
7. Faulkner SH, Spilsbury KL, Harvey J, Jackson A, Huang J, Platt M, Tok A, Nimmo MA (2014) The detection and measurement of interleukin-6 in venous and capillary blood samples, and in sweat collected at rest and during exercise. Eur J Appl Physiol 114:1207–1216. https://doi.org/10.1007/s00421-014-2851-8
8. Förster R, Schubel A, Breitfeld D, Kremmer E, Renner-Müller I, Wolf E, Lipp M (1999) CCR7 coordinates the primary immune response by establishing functional microenvironments in secondary lymphoid organs. Cell 99:23–33
9. Galen B, Cheshenko N, Tuyama A, Ramratnam B, Herold BC (2006) Access to nectin favors herpes simplex virus infection at the apical surface of polarized human epithelial cells. J Virol 80:12209–12218
10. Jones AP, Webb LM, Anderson AO, Leonard EJ, Rot A (1995) Normal human sweat contains interleukin-8. J Leukoc Biol 57:434–437
11. Kennedy WR, Sakuta M (1984) Collateral reinnervation of sweat glands. Ann Neurol 15:73–78
12. Lehman JS, Tollefson MM, Gibson LE (2009) Lichen planus. Int J Dermatol 48:682–694. https://doi.org/10.1111/j.1365-4632.2009.04062.x
13. Meller S, Gilliet M, Homey B (2009) Chemokines in the pathogenesis of lichenoid tissue reactions. J Invest Dermatol 2009 129:315–319. https://doi.org/10.1016/j.smim.2009.01.004
14. Mizukawa Y, Horie C, Yamazaki Y, Shiohara T (2012) Detection of varicella-zoster virus antigens in lesional skin of zosteriform lichen planus but not in that of linear lichen planus. Dermatology 225:22–26. https://doi.org/10.1159/000339771
15. Murakami M, Lopez-Garcia B, Braff M, Dorschner RA, Gallo RL (2004) Postsecretory processing generates multiple cathelicidins for enhanced topical antimicrobial defense. J Immunol 172:3070–3077
16. Nakai A, Hayano Y, Furuta F, Noda M, Suzuki K (2014) Control of lymphocyte egress from lymph nodes through β2-adrenergic receptors. J Exp Med 211:2583–2598. https://doi.org/10.1084/jem.20141132
17. Niyonsaba F, Suzuki A, Ushio H, Nagaoka I, Ogawa H, Okumura K (2009) The human antimicrobial peptide dermcidin activates

normal human keratinocytes. Br J Dermatol 160:239–243. https://doi.org/10.1111/j.1365-2133.2008.08925.x
18. Okiyama N, Fujimoto M (2015) Clinical perspectives and murine models of lichenoid tissue reaction/interface dermatitis. J Dermatol Sci 78:167–172. https://doi.org/10.1016/j.jdermsci.2015.03.001
19. 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 151:534–539
20. Rieg S, Steffen H, Seeber S, Humeny A, Kalbacher H, Dietz K, Garbe C, Schittek B (2005) Deficiency of dermcidin-derived antimicrobial peptides in sweat of patients with atopic dermatitis correlates with an impaired innate defense of human skin in vivo. J Immunol 174:8003–8010
21. Sallusto F, Lenig D, Mackay CR, Lanzavecchia A (1998) Flexible programs of chemokine receptor expression on human polarized T helper 1 and 2 lymphocytes. J Exp Med 187:875–883
22. Sato K, Sato F (1994) Interleukin-1 alpha in human sweat is functionally active and derived from the eccrine sweat gland. Am J Physiol 266(3 Pt 2):R950–R959
23. Schittek B, Hipfel R, Sauer B, Bauer J, Kalbacher H, Stevanovic S, Schirle M, Schroeder K, Blin N, Meier F, Rassner G, Garbe C (2001) Dermcidin: a novel human antibiotic peptide secreted by sweat glands. Nat Immunol 2:1133–1137
24. Shemer A, Weiss G, Trau H (2001) Wolf's isotopic response: a case of zosteriform lichen planus on the site of healed herpes zoster. J Eur Acad Dermatol Venereol 15:445–447
25. Shimoda-Komatsu Y, Sato Y, Hayashida Y, Yamazaki Y, Mizukawa Y, Nakajima K, Shiohara T, Aoyama Y (2017) Lichen amyloidosus as a sweat gland/duct-related disorder: resolution associated with restoration of sweating disturbance. Br J Dermatol 176: 1308–1315. https://doi.org/10.1111/bjd.15060
26. Shiohara T, Doi T, Hayakawa J (2011) Defective sweating responses in atopic dermatitis. Curr Probl Dermatol 41:68–79. https://doi.org/10.1159/000323297
27. Shiohara T, Mizukawa Y, Takahashi R, Kano Y (2008) Pathomechanisms of lichen planus autoimmunity elicited by cross-reactive T cells. Curr Dir Autoimmun 10:206–226. https://doi.org/10.1159/000131456
28. Sontheimer RD (2009) Lichenoid tissue reaction/interface dermatitis: clinical and histological perspectives. J Invest Dermatol 129:1088–1099. https://doi.org/10.1038/sj.jid.2009.42
29. Tan X, Li D, Wang X, Zeng Y, Yan Y, Yang L (2014) Clandin-2 downregulation by KSHV infection is involved in the regulation of endothelial barrier function. J Cutan Pathol 41:630–639. https://doi.org/10.1111/cup.12332
30. Ushigome Y, Sato Y, Shimoda Y, Yamazaki Y, Shiohara T (2017) Localized hypohidrosis is an unrecognized sequela of herpes zoster. J Am Acad Dermatol 76:160–162. https://doi.org/10.1016/j.jaad.2016.06.052
31. Vilches JJ, Navarro X (2002) New silicones for the evaluation of sudomotor function with the impression mold technique. Clin Auton Res 12:20–23
32. Villarroel VA, Okiyama N, Tsuji G, Linton JT, Katz SI (2014) CXCR3-mediated skin homing of autoreactive CD8 T cells is a key determinant in murine graft-versus-host disease. J Invest Dermatol 134:1552–1560. https://doi.org/10.1038/jid.2014.2
33. Wenzel J, Lucas S, Zahn S, Mikus S, Metze D, Ständer S, von Stebut E, Hillen U, Bieber T, Tűting T (2008) CXCR3 <-> lignand-mediated skin inflammation in cutaneous lichenoid graft-versus-host disease. J Am Acad Dermatol 58: 437–442. https://doi.org/10.1016/j.jaad.2007.10.647.
34. Wenzel J, Scheler M, Proelss J, Bieber T, Tűting T (2006) Type 1 interferon-associated cytotoxic inflammation in lichen planus. J Cutan Pathol 33:672–678
35. Wenzel J, Tüting T (2008) An IFN-associated cytotoxic cellular immune response against viral, self-, or tumor antigens is a common pathogenetic feature in interface dermatitis. J Invest Dermatol 128:2392–2402. https://doi.org/10.1038/jid.2008.96