Skin Barrier Dysfunction and Low Antimicrobial Peptide Expression in Cutaneous T-cell Lymphoma

Hiraku Suga,¹ Makoto Sugaya,¹* Tomomitsu Miyagaki,¹ Hanako Ohmatsu,¹ Makiko Kawaguchi,¹ Naomi Takahashi,¹ Hideki Fujita,¹ Yoshihide Asano,¹ Yayoi Tada,¹ Takafumi Kadono,¹ and Shinichi Sato¹

¹Department of Dermatology, Faculty of Medicine, University of Tokyo, Tokyo, Japan.

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*Address for reprint requests: Dr Makoto Sugaya, Department of Dermatology, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan.

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Abbreviations: MF, mycosis fungoides; SS, Sézary syndrome; CTCL, cutaneous T-cell lymphoma; Th2, T helper 2; AD, atopic dermatitis; AMP, antimicrobial peptide; hBD, human β defensin; TEWL, transepidermal water loss; mRNA, messenger RNA; mRNA, messenger RNA; SD, standard deviation; LIGHT, lymphotoxin-like, exhibits inducible expression, and competes with herpes simplex virus glycoprotein D for herpesvirus entry mediator, a receptor expressed by T lymphocytes; HVEM, herpesvirus entry mediator; STAT, signal transducer and activator of transcription
Translational Relevance

Skin is a barrier against pathogens such as bacteria, viruses, and fungi. Atopic dermatitis (AD) is characterized by decreased skin expression of filaggrin and loricrin, leading to abnormalities in skin barrier function and increased susceptibility to infections. Patients with cutaneous T-cell lymphoma (CTCL), which is a T helper 2-dominant disease like AD, often suffer from skin infection as well, leading us to investigate the skin barrier in CTCL. In this study, we have shown that messenger RNA levels of filaggrin and loricrin in lesional skin of advanced CTCL are decreased compared to normal skin, which negatively correlate with disease severity markers such as CCL17 and IL-22. Antimicrobial peptide expression in lesional skin of CTCL was significantly lower than in psoriatic skin. Identification of skin barrier dysfunction in CTCL patients, as shown here, may potentially lead to therapeutic strategies to prevent infections in these patients, leading to improved disease-associated morbidity.
Abstract

**Purpose:** Atopic dermatitis (AD) is characterized by decreased expression of filaggrin and loricrin. AD patients often suffer from skin infections, which are also frequently seen in patients with cutaneous T-cell lymphoma (CTCL). In this study, we aimed to investigate the skin barrier in CTCL.

**Experimental Design:** We assessed skin moisture and transepidermal water loss (TEWL) in patients with CTCL. We next examined messenger RNA (mRNA) expression levels of filaggrin, loricrin, and antimicrobial peptides (AMPs) in skin samples of CTCL, using skin from healthy volunteers and patients with AD or psoriasis as controls. Immunostainings for filaggrin, loricrin, and S100 proteins were also performed.

**Results:** Lower levels of skin moisture accompanied by higher levels of TEWL were seen in lesional skin of CTCL compared to normal skin. CTCL lesional skin contained lower levels of filaggrin and loricrin mRNA than normal skin, which was also true with AD and psoriatic skin. mRNA expression levels of filaggrin in CTCL skin negatively correlated with disease severity markers. Expression levels of AMPs in lesional skin of CTCL and AD were significantly lower than in psoriatic skin. Immunohistochemistry confirmed decreased expression of filaggrin and loricrin in CTCL, AD, and psoriatic skin, and enhanced expression of S100 proteins in psoriatic skin.

**Conclusions:** Our results show that there is barrier dysfunction in CTCL skin, similar to what
is seen with AD skin. In addition, low AMP expression in CTCL skin was documented when compared to psoriatic skin, which may explain frequent infections that can occur in CTCL patients.
Introduction

Mycosis fungoides (MF) and Sézary syndrome (SS) are the most common types of cutaneous T-cell lymphoma (CTCL). Patients with MF typically have a prolonged clinical course and only limited cases progress over years through patch, plaque, and tumor stages, followed by lymph node and visceral involvement (1). SS is characterized by the triad of generalized erythroderma (defined as affecting >80% of total body surface area), lymphadenopathy, and presence of more than 1,000 per mm$^3$ circulating atypical T-cells with cerebriform nuclei, so-called Sézary cells (2). Individuals with SS usually show rapid disease progression compared to MF patients. Most cases with MF/SS, especially at an advanced stage, show a T helper 2 (Th2)-dominant phenotype, characterized by increased IL-4, IL-5, IL-10, and IL-13 production (3, 4). Patients with CTCL often suffer from skin infections with bacteria, viruses, and fungi as well. Among them, *Staphylococcus aureus* is the most common infection in CTCL (5). In immunocompromised patients with advanced stage CTCL, skin infections occasionally lead to sepsis, multiple organ failure, and death (6, 7). The underlying basis for these infections has not been elucidated.

The epidermis provides an important physical barrier against environmental insults. Dry skin can cause many serious complications such as discomfort and itching, development of dermatitis, and bacterial and viral infections. Epidermal proteins such as filaggrin and loricrin are important in maintaining skin barrier function. Filaggrin aggregates keratin filaments and
provides a cytoskeleton for the cornified envelope (8). Loricrin is initially expressed in the granular layer and comprises 70% of the total protein mass of the cornified layer (8, 9). Skin barrier dysfunction is quite commonly seen in patients with atopic dermatitis (AD), which is caused by loss of function in these proteins (10, 11).

Antimicrobial peptides (AMPs) are part of the innate immune response and are found among all classes of life (12). These peptides kill bacteria, mycobacteria, enveloped viruses, and fungi. S100 family and human β defensin (hBD)-1, 2, and 3, which are all representative AMPs expressed by keratinocytes, are reported to be increased in psoriatic skin, probably due to increased expression of IL-17A and IL-22 (13-15). It is still controversial whether AMPs are increased or decreased in AD lesional skin (16-19). Although there have been considerable numbers of papers showing increased AMP expression in AD lesional skin, recent reports indicate that induction, release, and mobilization of AMPs in AD skin does not reach sufficient levels to provide adequate control of cutaneous microbial colonization (20).

Th2 cytokines appear to negatively influence the expression and induction of some AMPs (18, 19). Interestingly, a recent report shows that production of AMPs by keratinocytes in adult T-cell leukemia/lymphoma is reduced, leading to perturbed innate immunity and the frequent occurrence of superficial dermatophytosis (21).

In this study, we investigated skin barrier function and expression of AMPs in patients with CTCL, which shares many common immunologic features with AD. Specifically, we
assessed skin moisture, transepidermal water loss (TEWL), and expression of filaggrin, loricrin, S100 proteins, and defensins in CTCL patients. Skin from healthy volunteers and patients with AD or psoriasis were used as control samples. Taken together, our results suggest that skin barrier dysfunction occurs in CTCL patients, similar to what is seen in AD patients. These cutaneous defects likely explain the frequent occurrence of infections in CTCL patients, and may eventually lead to new strategies to improve disease-associated morbidity.
Materials and Methods

Patients and samples

Messenger RNA was obtained from biopsy materials of lesional skin of CTCL (n = 26, 17 males and 9 female; mean ± standard deviation (SD) age: 57.6 ± 12.3 years; 7 cases with patch MF, 8 cases with plaque MF, 5 cases with tumor MF, 2 cases with erythrodermic MF, and 4 cases with SS), AD (n = 6, all extrinsic type), and psoriasis (n = 5), and normal skin adjacent to benign skin tumors (n = 6) using RNeasy Fibrous Tissue Mini Kit (QIAGEN, Valencia, CA). All patients were either untreated or treated with only topical corticosteroids at the time of biopsy. The diagnosis of MF and SS and the stages of CTCL were based on clinical criteria as well as on histologic and immunohistochemical assessment according to World Health Organization classification and the criteria of the International Society for Cutaneous Lymphomas (2, 22). When classifying patients into patch MF, plaque MF, tumor MF, or erythrodermic MF/SS, the most severe skin lesion was taken into consideration. AD was diagnosed according to Hanifin and Rajka criteria (23). Healthy controls had no history of CTCL, AD, psoriasis, or any inflammatory skin diseases. Indeed, we specifically excluded patients under treatment for various internal and inflammatory disorders other than CTCL, AD, and psoriasis. All samples were collected after informed consent during daily clinical practice. The medical ethical committee of the University of Tokyo approved all described studies and the study was conducted according to the Declaration of Helsinki Principles.
We evaluated skin moisture and TEWL in lesional skin of CTCL patients (n = 11, 8 males and 3 females; 59.4 ± 11.3 years; 4 cases with patch MF, 2 cases with plaque MF, 3 cases with tumor MF, and 2 cases with erythrodermic MF/SS) and healthy volunteers (n = 8, 6 males and 2 females; 54.4 ± 20.3 years). Skin moisture and TEWL were evaluated at least 24 hours after the last application of moisturizers and/or topical corticosteroids as previously described (24, 25). CTCL lesions located in the lower abdominal area or equivalent normal skin areas in healthy volunteers were chosen as the test regions. Moreover, we examined skin moisture and TEWL in perilesional normal-appearing skin in the same CTCL patients. Perilesional skin was located at least 3 cm outside of the border of CTCL lesional skin. None of CTCL patients in this study had ichthyosis vulgaris. The measurements were performed in the months of April and May, when the humidity was 40-50% and the room temperature was kept at 20 °C.

First, we put water-soaked gauze on the test region. Next, we softly wiped the region with dry gauze. Five minutes later, we evaluated skin moisture and TEWL by using SKICON-200EX (IBS Co. Ltd., Hamamatsu, Japan) and Tewameter TM 300 (Courage & Khazaka, Köln, Germany), respectively. Skin moisture levels were displayed on the machine within one second after application of the probe onto the skin. Regarding TEWL levels, values were displayed on a recorder, and the mean value during the period 40 seconds after application of
the probe onto the skin was calculated. Skin moisture levels were evaluated five times in one skin lesion, and TEWL values were measured twice. Mean values were calculated.

**Quantitative reverse-transcriptase PCR assay**

Complementary DNA was synthesized using iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Berkeley, CA). Quantitative reverse transcriptase-PCR was performed as described previously based on SYBR Green assay (26). Primers for human filaggrin, loricrin, S100A7, S100A8, S100A9, hBD-1, hBD-2, hBD-3, and GAPDH were as follows: filaggrin forward, 5’-GAA GAC AAG GAT CGC ACC AC-3’ and reverse, 5’-ATG GTG TCC TGA CCC TCT TG-3’; loricrin forward, 5’-TCA TGA TGC TAC CCG AGG TTT G-3’ and reverse, 5’-CAG AAC TAG ATG CAG CCG GAG A-3’; S100A7 forward, 5’-CTT CCT TAG TGC TAC TGA TCC AGG TTT G-3’ and reverse, 5’-AAA GAC AGA AAC TCA GAA AAA TCA ATC T-3’; S100A8 forward, 5’-ATG CCG TCT ACA GGG ATG AC-3’ and reverse, 5’-ACG CCC ATC TTT ATC ACC AAG-3’; S100A9 forward, 5’-CAG CTG GAA CGC AAC ATA GA-3’ and reverse, 5’-TCA GCT GCT TGT CTG CAT TT-3’; hBD-1 forward, 5’-AGA TGG CCT CAG GTG GTA ACT TT-3’ and reverse, 5’-GGG CAG GCA GAA TAG AGA CAT T-3’; hBD-2 forward, 5’-GAT GCC TCT TCC AGG TGT TTT T-3’ and reverse, 5’-GGA TGA CAT ATG GCT CCA CTC TT-3’; hBD-3 forward, 5’-GTG AAG CCT AGC AGC TAT GAG GAT-3’ and reverse, 5’-TGA TTC CTC CAT GAC CTG GAA-3’; GAPDH forward, 5’-ACC CAC TCC
TCC ACC TTT GA-3’ and reverse, 5’-CAT ACC AGG AAA TGA GCT TGA CAA-3’.

**Immunohistochemistry**

We performed immunohistochemical staining for filaggrin, loricrin, S100A7, and S100A8 with lesional skin of 20 cases of CTCL [patch MF (n = 5), plaque MF (n = 5), tumor MF (n = 5), erythrodermic MF/SS (n = 5)], AD (n = 5), and psoriasis (n = 5). Normal skin adjacent to benign skin tumors served as controls (n = 5). Briefly, 5 µm-thick tissue sections from formaldehyde-fixed and paraffin-embedded samples were de-waxed and rehydrated. These sections were then stained with mouse anti-human filaggrin monoclonal antibody (Santa Cruz Biotech, California, CA), rabbit anti-human loricrin polyclonal antibody (Santa Cruz), mouse anti-human S100A7 monoclonal antibody (Santa Cruz), or mouse anti-human S100A8 monoclonal antibody (Santa Cruz), followed by ABC staining (Vector Lab, Burlingame, CA). Diaminobenzidine was used for visualizing the staining, and counterstaining with Mayer hematoxylin was performed, according to manufacturers’ instructions.

**Statistical analyses**

Statistical analyses were performed using the Mann-Whitney’s U-test and Student’s t-test for comparison of two groups. For testing equality of population means among three or more groups, Kruskal-Wallis test and Scheffe’s F test were used. Correlation coefficients were
determined by using the Spearman’s rank correlation test. $P$-values of $< 0.05$ were considered statistically significant.
Results

Lower levels of skin moisture accompanied by higher levels of TEWL in lesional skin of CTCL

To investigate skin barrier function in CTCL patients, we evaluated skin moisture and TEWL in lesional and non-lesional skin of CTCL and in normal skin. Skin moisture levels in CTCL lesional skin were significantly lower than those found in non-lesional skin and normal skin (Fig. 1, $P < 0.01$, each). Moreover, skin moisture levels in non-lesional skin from CTCL patients were lower than normal control skin (Fig. 1, $P < 0.01$).

TEWL values in CTCL lesional skin were significantly higher those in non-lesional skin and normal skin (Fig. 1, $P < 0.01$, each). There were also significant differences in TEWL values between non-lesional CTCL skin and normal control skin (Fig. 1, $P < 0.05$). Thus, skin barrier function was abnormal in both lesional and non-lesional skin of CTCL patients when compared to normal control skin.

Decreased mRNA expression of filaggrin and loricrin combined with increased mRNA expression of S100 family and hBD-1 in CTCL skin

We next analyzed expression levels of skin barrier-associated proteins and AMPs in lesional skin of CTCL, AD, psoriasis, and healthy individuals. Filaggrin mRNA expression levels in lesional skin of plaque, tumor, or erythroderma of CTCL were significantly decreased
compared to normal skin (Fig. 2). Similar results were obtained with regard to loricrin mRNA. Filaggrin and loricrin mRNA expression levels in lesional skin of AD and psoriasis were also decreased compared to normal controls (Fig. 2), which was consistent with previous reports (10, 11). S100A7 mRNA expression levels in lesional skin of patch, plaque, tumor, erythroderma of CTCL, AD, and psoriasis were significantly higher than in normal controls (Fig. 2; P < 0.05, P < 0.01, P < 0.05, P < 0.01, P < 0.01, and P < 0.05, respectively).

Similarly, lesional skin of plaque, tumor, erythroderma of CTCL, AD, and psoriasis expressed significant higher levels of S100A8 mRNA than in normal skin (Fig. 2; P < 0.01, P < 0.05, P < 0.05, P < 0.01, and P < 0.05, respectively). S100A9 mRNA expression levels in lesional skin of plaque, tumor of CTCL, and psoriasis were significantly higher than normal controls (Fig. 2; P < 0.01, P < 0.05, and P < 0.05, respectively). With regard to hBD-1, in lesional skin of plaque, tumor, erythroderma of CTCL, and AD, significantly decreased mRNA expression levels were detected compared to normal controls (Fig. 2; P < 0.05, each).

Although previous reports showed that hBD-1 expression correlated with disease activity in psoriasis (15, 27), it was not elevated in lesional skin of psoriasis compared to normal skin. Of note, mRNA levels of S100A7 and S100A8 in CTCL lesional skin were significantly decreased as compared with levels in psoriatic skin (Fig. 2; P < 0.01, each). There were no significant differences in hBD-2 and hBD-3 expression among the groups (data not shown).

Thus, filaggrin and loricrin mRNA expression levels were decreased in CTCL lesional skin
combined with increased S100 family protein expression, although the latter was not as high as seen in psoriatic skin.

**Correlations between filaggrin expression in lesional skin of CTCL and disease activity**

We evaluated correlations between expression levels of filaggrin and those of loricrin, S100 family, hBD-1, hBD-2, hBD-3, and disease severity markers in CTCL lesional skin. We have previously reported that expression levels of CCL17, CCL18, CCR4, IL-4, and IL-22 are associated with disease progression in CTCL (3, 28-31). LIGHT [lymphotoxin-like, exhibits inducible expression, and competes with HSV glycoprotein D for herpesvirus entry mediator (HVEM), a receptor expressed by T lymphocytes] is also correlated with disease progression in CTCL, while HVEM expression in CTCL skin negatively correlates with disease progression (28). Filaggrin expression levels positively correlated with those of loricrin (Fig. 3A), which is consistent with the fact that both molecules are barrier function-related proteins down-regulated by Th2 cytokines (32, 33). Filaggrin expression levels negatively correlated with those of CCL17, CCL18, CCR4, IL-4, IL-22, and LIGHT, and positively correlated with those of HVEM (Fig. 3A), suggesting a significant negative correlation between disease progression in CTCL and filaggrin expression. Moreover, we evaluated associations between filaggrin expression in CTCL lesional skin and serum levels of sIL-2R or CCL17 (29, 31). Interestingly, there were significant negative correlations between filaggrin expression and
serum levels of these disease markers (Fig. 3B). Thus, as severity of CTCL progresses, expression of skin barrier-related proteins decreases in lesional skin.

**Correlations between S100A7 expression in lesional skin of CTCL and disease activity**

We next evaluated correlations between expression levels of S100A7, a representative AMP (34), and those of loricrin, S100A8, S100A9, hBD-1, hBD-2, hBD-3, and disease severity markers in CTCL lesional skin. As expected, S100A8 and S100A9 expression levels positively correlated with S100A7 expression levels (Fig. 4A). Expression levels of CCL26 and CCR3, which is a prototypic Th2 chemokine/chemokine receptor pair, negatively correlated with those of S100A7 (Fig. 4A). We have reported that expression levels of CCL26 and CCR3 are increased in lesional skin of advanced CTCL compared to normal controls (29, 35, 36). Thus, when disease progresses, production of AMPs in CTCL lesional skin, which is abundant in Th2 cytokines and chemokines, decreases. In turn, this likely leads to the frequent occurrence of skin infections seen in advanced CTCL patients.

**No correlation between S100A7 and IL-17A or IL-22 expression in CTCL lesional skin**

Expression of AMPs including S100A7, hBD-1, and hBD-2 by keratinocytes is increased by stimulation with IL-17A and/or IL-22 *in vitro* (13, 14). Expression of these cytokines is up-regulated in psoriatic skin compared to normal skin (37, 38). We have reported that IL-22,
but not IL-17A, is up-regulated in CTCL lesional skin (27). Therefore, we examined correlations between S100A7 expression and IL-17A or IL-22 expression in CTCL lesional skin. There were no positive correlations between expression levels of S100A7 and either IL-17A or IL-22 in CTCL lesional skin (Fig. 4B). Thus, AMP expression was not increased in proportion to IL-17A or IL-22 expression in CTCL skin, probably because Th2 cytokines, abundantly expressed in advanced CTCL skin, blocked AMP expression by lesional keratinocytes as was reported in vitro (18, 19).

**Decreased filaggrin and loricrin expression by keratinocytes in lesional skin of CTCL, AD, and psoriasis and enhanced S100A7 and S100A8 expression in psoriatic skin**

Immunohistochemical stainings for filaggrin, loricrin, S100A7, and S100A8 were performed using lesional skin of patch, plaque, tumor, erythroderma of CTCL, AD, psoriasis, and normal skin. In patch and plaque CTCL cases, filaggrin and loricrin expression by keratinocytes was slightly decreased compared to normal skin (Fig. 5 and Table I). In almost all cases with tumor and erythroderma of CTCL, AD, and psoriasis, filaggrin and loricrin expression was remarkably decreased (Fig. 5 and Table I). S100A7 and S100A8 expression was remarkably enhanced only in psoriatic skin. These immunohistochemical findings were largely consistent with mRNA levels detected by real-time PCR (Fig. 2). Thus, expression of skin barrier-related proteins by keratinocytes in lesional skin of CTCL, AD, and psoriasis was
decreased, whereas AMPs were highly produced by keratinocytes of lesional skin of psoriasis, but not in CTCL or AD skin.
Discussion

Our study revealed that skin moisture levels were decreased and TEWL was increased in lesional skin of CTCL compared to normal skin. Lesional skin of advanced CTCL also expressed lower levels of filaggrin mRNA, which negatively correlated with mRNA levels of disease severity markers. Expression levels of AMPs in lesional skin of CTCL and AD were significantly lower than those in psoriatic skin, which may explain the frequent occurrence of cutaneous infections in CTCL patients.

Measuring skin moisture and TEWL are non-invasive means to assess barrier function of the stratum corneum. Previously, skin moisture and TEWL were evaluated with various skin diseases such as AD, ichthyosis, and psoriasis, all of which are strongly associated with dry skin (24, 25). To the best of our knowledge, this is the first study to evaluate skin moisture and TEWL in CTCL patients. Of note, to control for any age-related changes in skin function (39), we utilized normal volunteers of the approximate same age as the CTCL patients. Skin moisture levels in lesional skin of CTCL were significantly lower than those in normal skin, while TEWL in CTCL skin was significantly higher than in normal skin (Fig. 1). Moreover, lower levels of skin moisture combined with higher levels of TEWL were detected in CTCL perilesional skin compared to normal skin. Although we did not evaluate perilesional skin histologically in this study, atypical T-cell infiltration is often seen in normal-appearing skin of CTCL patients. Therefore, direct infiltration of tumor cells as well as Th2-dominant
systemic inflammation can cause skin barrier dysfunction in non-lesional skin of CTCL. Thus, we clearly show here that CTCL patients have dry skin, which can subsequently lead to pain, pruritus, and infections.

The recent identification and confirmation of loss-of-function mutations in filaggrin as a major risk factor for AD sheds new light on the immunopathogenesis of this disease (10, 11). Skin barrier dysfunction and resultant diminished epidermal defense mechanisms to allergens and microbes are regarded as early steps in the onset of AD (16-19). Th2-dominant immune responses such as increased levels of IgE may result from, rather than cause, refractory eczema as has been reported in mice repeatedly exposed to cutaneous allergens (40). A recent report, however, showed that expression of filaggrin and loricrin by primary human keratinocytes was inhibited by adding IL-4, IL-13, or the combination of IL-4 and IL-13 (33). Skin biopsies from signal transducer and activator of transcription (STAT)6 transgenic mice were deficient in loricrin and involucrin, suggesting that Th2 cytokines such as IL-4 and IL-13 inhibit expression of barrier function-related proteins through a STAT6-dependent mechanism (33). IL-4 and IL-13, which are highly expressed in skin and blood of patients with either AD or CTCL (30, 41, 42), may in turn down-regulate filaggrin and loricrin expression in these patients. Indeed, we found significant negative correlations between filaggrin expression and disease severity markers (including IL-4) in CTCL lesional skin (Fig. 3A). On the other hand, expression of filaggrin and loricrin were also decreased in lesional
skin of psoriasis patients (Fig. 2), which is consistent with previous reports (13-15). Tumor necrosis factor-α, expressed in psoriatic skin, down-regulates filaggrin and loricin expression \textit{in vitro}. Our results, along with previous reports, suggest that decreased expression of filaggrin is not a specific finding of AD, since this was readily seen in CTCL and psoriasis patients as well.

While CTCL, AD, and psoriasis are all T cell–associated diseases that share common features such as epidermal hyperplasia, abundant inflammatory cell infiltrates, and decreased expression of filaggrin, the two former diseases have different immune and barrier phenotypes from the latter. AD is a Th2/Th22-polarized disease with an attenuated Th17 axis (43). Expression of IL-22 and Th2 cytokines such as IL-4 and IL-10, but not IL-17A, is also elevated in lesional skin of CTCL (30). By contrast, IL-17A and IL-22 are elevated in psoriatic skin, but not Th2 cytokines. Th2 cytokines inhibit secretion of AMPs such as S100A7, hBD-2, and hBD-3 in primary keratinocytes (44-46). Consistently, in CTCL lesional skin, we detected significant negative correlations between expression levels of S100A7 and those of CCL26 or CCR3, a representative Th2 chemokine/chemokine receptor pair (Fig. 4A). Th2 cytokines also suppress Th1 cytokines, which are effective for anti-tumor immunity and infections (47, 48). Thus, a Th2-dominant cytokine milieu down-regulates immunity against infections, which are commonly seen in lesional skin of CTCL as well as in AD skin. Not
surprisingly, psoriasis patients do not have clinical issues with skin infections, since Th2 cytokines are not dominant.

We also showed that expression levels of AMPs were higher in psoriatic skin than in CTCL or AD skin (Fig. 2). IL-17A, together with IL-22, induces AMPs such as S100A7, S100A8, S100A9, and hBD-2 (49). Interestingly, there were positive correlations between expression levels of S100A7 and those of IL-17A or IL-22 in psoriatic skin (data not shown), whereas no such correlations were found in CTCL skin (Fig. 4B). Since Th2 cytokines suppress expression of AMPs as well as IL-17A, high expression of Th2 cytokines may be the main reason why AMP levels were decreased in CTCL and AD compared with psoriasis.

In the skin of erythrodermic CTCL patients, *Staphylococcus aureus*-derived superantigen enterotoxins are commonly found, which could exacerbate chronic expansion of T cells, including T-cell receptor Vβ2-bearing cells (5, 50). Therefore, insufficient induction of AMPs, especially S100A8 and S100A9, may cause infection with staphylococcus in CTCL skin, which may induce expansion of T cells bearing specific T-cell receptors. Although psoriatic skin is often colonized with *Staphylococcus aureus*, life-threatening infection is rarely seen probably due to sufficient expression of AMPs.

In conclusion, our study has revealed that skin barrier dysfunction is present in CTCL, similar to what has been reported in AD. Th2/Th22-polarized immune status together with an attenuated Th17 axis may cause decreases in filaggrin expression and insufficient induction
of AMPs. Thus, improving skin barrier function by attenuating Th2 responses could lead to better control of cutaneous infections, improving overall disease-associated morbidity in patients with CTCL.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

H. Suga carried out research and analyzed data. M. Sugaya designed the research and wrote the paper. T. Miyagaki, M. Kawaguchi, N. Takahashi, and H. Ohmatsu collected clinical samples and data. H. Fujita, Y. Asano, Y. Tada, and T. Kadono contributed to the design of the research. S. Sato financially supported and helped design the research.

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References


7. Axelrod PI, Lorber B, Vonderheid EC. Infections complicating mycosis


15. Kim BE, Howell MD, Guttman-Yassky E, Guttman E, Gilleaudeau PM,


27. Gambichler T, Skrygan M, Tomi NS, Othlinghaus N, Brockmeyer NH, Altmeyer


39. Choi JW, Kwon SH, Huh CH, Park KC, Youn SW. The influences of skin


44. Gläser R, Meyer-Hoffert U, Harder J, Cordes J, Wittersheim M, Kobliakova J,


Table I. Filaggrin, loricrin, S100A7, and S100A8 expression in lesional skin of patch, plaque, tumor, erythroderma of cutaneous T-cell lymphoma, atopic dermatitis, psoriasis and in normal skin.

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CTCL, cutaneous T-cell lymphoma; AD, atopic dermatitis
Figure Legends

**Figure 1.** Skin moisture and transepidermal water loss (TEWL) in lesional and non-lesional skin from cutaneous T-cell lymphoma (CTCL) patients, and in normal control skin. The measured values from individual patients were plotted by dots. *P < 0.05. **P < 0.01.

**Figure 2.** mRNA expression of filaggrin, loricrin, S100A7, S100A8, S100A9, and human β defensin (hBD)-1 in lesional skin of patch, plaque, tumor, erythroderma of cutaneous T-cell lymphoma (CTCL), atopic dermatitis (AD), and psoriasis, and in normal skin. Each histogram shows the mean + SEM. **P < 0.05, **P < 0.01 versus normal skin. † P < 0.05, †† P < 0.01 versus psoriasis.

**Figure 3.** Correlations between filaggrin expression in lesional skin of cutaneous T-cell lymphoma (CTCL) and disease activity. A, significant positive or negative correlations between expression levels of filaggrin in CTCL lesional skin and those of loricrin or disease markers. B, significant negative correlations between filaggrin expression in CTCL lesional skin and serum soluble IL-2 receptor or CCL17 levels. The measured values from individual patients were plotted by dots.
Figure 4. Correlations between S100A7 expression in lesional skin of cutaneous T-cell lymphoma (CTCL) and disease activity. A, significant positive or negative correlations between expression levels of S100A7 and those of S100A8, S100A9, CCL26, or CCR3 in CTCL lesional skin. B, no significant correlations between expression levels of S100A7 and those of IL-17A or IL-22 in CTCL lesional skin. The measured values from individual patients were plotted by dots.

Figure 5. Immunohistochemical staining for filaggrin, loricrin, S100A7, and S100A8 in lesional skin of patch, plaque, tumor, and erythroderma of cutaneous T-cell lymphoma (CTCL), atopic dermatitis (AD), psoriasis, and in normal skin (original magnification x 400). Representative pictures of five cases in each group. -, no expression; +, modest expression; ++, high expression.
Skin moisture

(normal) 

CTCL

(normal) 

CTCL

FIGURE 1 Suga et al.
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Skin Barrier Dysfunction and Low Antimicrobial Peptide Expression in Cutaneous T-cell Lymphoma

Hiraku Suga, Makoto Sugaya, Tomomitsu Miyagaki, et al.

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