

Atopic Dermatitis-like Graft-versus-host Disease and Lichen Planus-like Graft-versus-host Disease: Alterations in Skin Barrier Function and Related Molecules

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Abstract

Background: Graft-versus-host disease (GVHD) is a common complication of hematopoietic stem cell transplantation. Skin barrier disruption could induce thymic stromal lymphopoietin (TSLP) expression, and the expression of TSLP was increased in lesions of atopic dermatitis (AD)-like GVHD and lichen planus (LP)-like GVHD. This study attempted to investigate the skin barrier function of AD-like GVHD and LP-like GVHD and possible mechanisms.

Methods: Eighteen AD-like GVHD patients, 12 LP-like GVHD patients, and 14 healthy volunteers were enrolled in this study. Skin biopsy was done in five AD-like GVHD patients, eight LP-like GVHD patients, and eight healthy volunteers. The intensity of pruritus was assessed by visual analog scale itch score and detailed pruritus score. Transepidermal water loss (TEWL) was measured using Tewameter[®] TM 300. Immunohistochemistry was used to observe the expression of loricrin, involucrin, LL37, and human β -defensins 2 (hBD2) in skin lesions. Western blot analysis was used for analyzing the protein levels of loricrin and involucrin in skin lesions. Real-time polymerase chain reaction was performed to assess the mRNA levels of LL37 and hBD2 in skin lesions.

Results: Pruritus score was higher in patients with AD-like GVHD (11.33 ± 5.35) than that of patients with LP-like GVHD (2.58 ± 3.09 , $P < 0.001$). Compared with healthy controls (HCs, $4.52 \pm 1.24 \text{ g} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$), TEWL was increased in AD-like GVHD ($26.72 \pm 9.02 \text{ g} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$, $P < 0.001$) and LP-like GVHD patients ($18.78 \pm 4.57 \text{ g} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$, $P < 0.001$), and expressions of loricrin and involucrin were also increased in skin lesions of AD-like GVHD and LP-like GVHD patients (all $P < 0.05$). LL37 mRNA expression was decreased in lesions of AD-like GVHD and LP-like GVHD patients ($P = 0.005$ and $P = 0.008$, vs. HCs, respectively). hBD2 mRNA expression was increased in skin lesions of AD-like GVHD and LP-like GVHD patients ($P = 0.002$ and $P < 0.001$, vs. HCs, respectively).

Conclusions: Skin barrier dysfunction is present in AD-like GVHD and LP-like GVHD. The immunoreactions, but not the congenital defect, are considered to be the primary cause of skin barrier impairment in AD-like GVHD and LP-like GVHD.

Key words: Atopic Dermatitis; Graft-versus-host Disease; Lichen Planus; Skin Barrier

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is a therapeutic option for various hematological malignancies, solid tumors, and severe immunodeficiency disorders. Graft-versus-host disease (GVHD) is a common complication of HSCT. GVHD has been classified into acute GVHD and chronic GVHD (cGVHD) based on clinical presentation and time of disease onset. cGVHD is a delayed complication in patients undergoing HSCT, which commonly affects the skin, eyes, gastrointestinal tract, liver, and lungs.^[1] The incidence of cGVHD ranges from 6% to 80%. Skin is the most common organ affected in cGVHD (75%).^[2] Cutaneous

cGVHD has various manifestations, mimicking a wide variety of autoimmune and inflammatory skin diseases, especially lichen planus (LP)-like GVHD and scleroderma (sclerodermoid GVHD).^[3] Poikiloderma-like, psoriasis-like, and dermatomyositis-like GVHD have also been reported.^[3-5]

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Our previous study had reported atopic dermatitis (AD)-like GVHD characterized by eczematous dermatitis, itching, dry skin, increase of peripheral blood eosinophils, and/or elevated serum IgE level with good prognosis.^[6]

AD is a common chronic inflammatory skin disease. The impairment of skin barrier function and abnormal immunoreactions are considered to be main possible mechanism of AD. The skin barrier is made up of several layers of keratinocytes and lipid matrix, consisting of water-retaining ceramides, cholesterol, and sphingosines. The outermost layers of the stratified epidermis are composed of tough cornified cells, and such cells are encapsulated within a highly specialized structure called cornified envelope. The cornified envelope is composed predominantly of proteins, including filaggrin, loricrin, involucrin, and other proteins.^[7,8] The skin can not only form a permeability barrier but also form an antimicrobial barrier with antimicrobial peptides (AMPs). AMPs are an evolutionarily conserved component of the innate immune system and have been shown to link the innate and adaptive immune responses. In humans, there are two major families of AMPs: defensins and cathelicidins. These peptides can either be constitutively expressed or inducible in various tissues and cell types following microbial challenge, the production of inflammatory mediators and injury. In addition, AMPs also contribute to maintenance the skin barrier function.^[9]

Skin barrier disruption could induce the expression of thymic stromal lymphopoietin (TSLP).^[10] Based on the results of our previous study, the expression of TSLP was increased in skin lesions of AD-like GVHD and LP-like GVHD patients. This study attempted to investigate the skin barrier function and related molecules in patients with AD-like GVHD and LP-like GVHD.

METHODS

Ethical approval

The study was conducted in accordance with the *Declaration of Helsinki* and was approved by the Ethics Committee of Peking University People's Hospital. All patients and healthy controls (HCs) gave written informed consent and participated voluntarily.

Patients and healthy controls

Eighteen patients with AD-like GVHD, 12 patients with LP-like GVHD, and 14 healthy volunteers were enrolled between May 2015 and January 2017, at Peking University People's Hospital, Beijing, China. Skin biopsy was done in five patients with AD-like GVHD, eight patients with LP-like GVHD and eight healthy volunteers. The diagnosis of AD-like GVHD was based on the diagnostic criteria proposed by Wei *et al.*,^[6] and the diagnosis of LP-like GVHD was made according to the National Institutes of Health Consensus criteria.^[3]

Pruritus severity assessment

The visual analog scale itch score and detailed pruritus score (DPS) proposed by Duo^[11] were used to assess severity of pruritus, which is based on a combined score of severity

and distribution of pruritus and sleep disturbance. Sleep disturbance and severity/distribution scores were added up to calculate the patient's final score. The evaluation criteria of DPS are shown in Table 1.

Transepidermal water loss

Transepidermal water loss (TEWL) was measured using Tewameter[®] TM 300, an open chamber device (Courage+Khazaka Electronic GmbH, Cologne, Germany) and expressed in $\text{g}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$. The Tewameter[®] TM 300 has accuracy of $\pm 0.5 \text{ g}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ under normal room condition (10–30°C) and humidity 40–60% according to the manufacturer's instruction.

Skin samples

The skin samples were obtained for biopsy and divided into three parts. One part was fixed with 4% paraformaldehyde for histopathological and immunohistochemistry study. The other two parts were stored in liquid nitrogen for extracting total protein and total RNA separately.

Histopathology and Immunohistochemistry

The formalin-fixed skin tissues were embedded in paraffin and cut into 5 μm thick sections and stained with hematoxylin and eosin.

For immunohistochemistry, the section slices were deparaffinized and incubated in 3% hydrogen peroxide for 10 min. After washing three times with phosphate-buffered saline (PBS) for 5 min, the sections were boiled in citrate buffer (pH 6.0) for 15 min using a microwave oven to heat-induced antigen retrieval. Then, the sections were washed in PBS and blocked with 5% bovine serum albumin for 30 min. Subsequently, the sections were incubated with the following primary antibody: rabbit anti-loricrin antibody (1:200, Abcam, Cambridge, UK), rabbit anti-involucrin antibody (1:100, Abcam, Cambridge, UK), rabbit anti-LL37 antibody (1:500, Abcam, Cambridge, UK), and rabbit anti-beta 2 defensin antibody (1:500, Abcam, Cambridge, UK) overnight

Table 1: The evaluation criteria of detailed pruritus score

Pruritus characteristics	Score
Severity of pruritus	
Mild need for scratching	1
Need for scratching without excoriation	2
Need for scratching with excoriation	4
Frustrating pruritus	5
Distribution of pruritus	
<2 sites	1
≥2 sites	2
Generalized	3
Sleep disturbance as a result of pruritus	
Waking up of pruritus	1 for each time per night, up to 10
Scratching during night with excoriation	1 for each time per night, up to 5

at 4°C in a wet chamber. After washing three times with PBS for 5 min, the sections were stained with goat anti-mouse/rabbit secondary antibody for 30 min at room temperature and stained with diaminobenzidine, then counterstained using hematoxylin. All slides were recorded using the digital Leica Application Suite imaging system (Leica, Solms, Germany).

Western blotting analysis

The skin tissues were lysed with RIPA Lysis Buffer (Solarbio, Beijing, China) using TissueLyser II (Qiagen, CA, USA) with shaking at 20 Hz for 5 min and placing on ice for 5 min. The aforementioned procedure was repeated twice. The lysates were centrifuged at 12,000 ×g for 15 min. Protein concentrations were determined using the BCA assay (Thermo, IL, USA). The protein samples were boiled for 5 min at 100°C and put on ice. Subsequently, an equal amount of protein sample was loaded in each well and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis for electrophoresis and then transferred onto polyvinylidene difluoride (PVDF) membrane. After blocking with 5% skim milk for 1 h at room temperature, the PVDF membrane was incubated overnight at 4°C with the following primary antibodies: rabbit anti-loricrin antibody (1:1000, Abcam, Cambridge, UK) and rabbit anti-involucrin antibody (1:1000, Abcam, Cambridge, UK). Mouse-anti-β-actin (1:1000, Proteintech, IL, USA) was used as the controls. On the following day, the membrane was washed three times with TBST for 10 min and then incubated with corresponding secondary antibodies for 1 h at room temperature. Finally, specific bands were observed by ImageQuant 350 (GE, MA, USA) with standard chemiluminescence (Thermo, IL, USA). The band intensity was calculated by ImageJ software (National Institutes of Health, MD, USA) compared to β-actin.

Reverse transcription and real-time polymerase chain reaction

The skin tissues were put into buffer RLT (Qiagen RNeasy Fibrous Tissue Mini Kit, CA, USA) and homogenized with Qiagen TissueLyser II (Qiagen, CA, USA) at 20 Hz vibrating for 5 min twice. Total RNA was extracted according to the manufacturer's instruction. The RNA concentration was determined with a NanoVue Plus Spectrophotometer (GE, MA, USA). The cDNA was synthesized in 10 μl reaction systems by a reverse transcription kit (Toyobo, Osaka, Japan). The reactions were amplified and quantified by Power SYBR Green RT-PCR Reagents Kit (Applied Biosystems, CA, USA) on a 7500 Fast Real-time PCR Amplifier (Applied Biosystems, CA, USA). The protocol included holding stage at 95°C for 10 min, followed by cycling stage with 40 cycles of amplification at 95°C for 15 s and 60°C for 1 min. Glyceraldehyde-3-phosphate dehydrogenase was used to normalize each sample and each gene. The primer sequences (Sangon, Beijing, China) are summarized in Table 2. Relative expression ratio was calculated using the comparative threshold cycle (Ct) and $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

All data were conducted by SPSS version 21.0 (IBM Corp.,

NY, USA) for statistical analysis, and GraphPad Prism software (GraphPad, CA, USA) was used to plot the graphs. The comparisons between the two groups were analyzed using Mann-Whitney *U*-test and Student's *t*-test. One-way analysis of variance was used to multiple comparisons. A *P* < 0.05 was considered statistically significant.

RESULTS

Patients' characteristics

Characteristics of patients with AD-like GVHD and LP-like GVHD are summarized in Table 3. The striking clinical features in patients with AD-like GVHD included eczematous dermatitis, dry skin, pronounced itching and perifollicular accentuation, and the features of LP-like GVHD patients included erythematous/violaceous flat-topped papules or plaques with or without surface reticulations or a silvery or shiny appearance [Figure 1a and 1b]. In addition, the score of pruritus was significantly higher in patients with AD-like GVHD (11.33 ± 5.35) than that in patients with LP-like GVHD (2.58 ± 3.09 , $t = 5.111$, $P < 0.001$).

Pathological features of atopic dermatitis-like graft-versus-host disease and lichen planus-like graft-versus-host disease

For histopathological features of AD-like GVHD patients, the epidermal changes included hyperkeratosis, parakeratosis,

Table 2: Primers for PCR amplification of genes used in this study

Genes	Primer sequence
GAPDH	Forward: 5'-GGTGAAGGTCGGTGTGAACG-3' Reverse: 5'-CTCGCTCCTGGAAGATGGTG-3'
LL37	Forward: 5'-GCAGTCACCAGAGGATTGTGAC-3' Reverse: 5'-CACCGCTTCACCAGCTG-3'
hBD2	Forward: 5'-TGTGGTCTCCCTGGAACAAAAT-3' Reverse: 5'-GTCGCACGTCTCTGATGAGG-3'

GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; hBD2: Human β-defensins 2; PCR: Polymerase chain reaction.



Figure 1: Clinical presentations of patients with AD-like GVHD and LP-like GVHD. (a) Lesions of AD-like GVHD showed the papules and excoriation in legs. (b) Lesions of LP-like GVHD showed the flat-topped papules with surface reticulations or a silvery or shiny appearance in hands. AD: Atopic dermatitis; GVHD: Graft-versus-host disease; LP: Lichen planus.

Table 3: Characteristics of patients with AD-like GVHD and LP-like GVHD

Characteristics	Patients with AD-like GVHD (n = 18)	Patients with LP-like GVHD (n = 12)
Gender, n		
Male	9	10
Female	9	2
Age (years), mean ± SE	25.8 ± 9.9	29.8 ± 9.5
Original diseases, n		
AML	6	4
CML	1	2
ALL	9	4
MDS	1	2
AA	1	0
Donor/recipient, n		
Father/daughter	4	0
Father/son	4	3
Mother/son	1	1
Daughter/father	0	1
Daughter/mother	1	0
Son/father	0	2
Sister/brother	4	2
Brother/sister	3	2
Uncle/nephew	0	1
Unrelated	1	0
Mean time after HSCT (months), mean ± SE	16.4 ± 12.9	23.5 ± 12.0
Onset of skin lesion after HSCT (day), median (IQR)	175.0 (118.5)	375.0 (582.0)
History of allergic disease of donor, n	6	0
Dry skin	16	9
Distribution of lesions, n		
Face	0	2
Palms or soles	0	2
Face and trunk	1	0
Face, trunk, and extremities	2	1
Trunk and extremities	2	3
Widespread	13	4
Nails	2	7
Oral mucosa	2	8
Other system involvements, n		
No	13	3
Yes	5	9
Skin biopsy	5	8

AML: Acute myeloid leukemia; CML: Chronic myeloid leukemia; ALL: Acute lymphoid leukemia; MDS: Myelodysplastic syndrome; AA: Aplastic anemia; IQR: Interquartile range; GVHD: Graft-versus-host disease; AD: Atopic dermatitis; LP: Lichen planus; HSCT: Hematopoietic stem cell transplantation; SE: Standard error.

epidermal spongiosis, and scattered keratinocyte necrosis. The dermal changes often showed a sparse perivascular lymphocytic infiltrate and eosinophils infiltrate. The histopathological features of LP-like GVHD patients included hyperkeratosis, focal increases in the granular cell layer, vacuolar changes of basal layer, scattered keratinocyte necrosis in the epidermis and melanophages, and a large number of lymphocytic infiltration in the dermis [Figure 2].

Transepidermal water loss in patients with atopic dermatitis-like graft-versus-host disease and lichen planus-like graft-versus-host disease

Compared with HCs ($4.52 \pm 1.24 \text{ g} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$), TEWL were significantly increased in patients with AD-like GVHD ($26.72 \pm 9.02 \text{ g} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$, $U = 0.000$, $P < 0.001$) and LP-like GVHD ($18.78 \pm 4.57 \text{ g} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$, $U = 0.000$, $P < 0.001$). Moreover, TEWL in patients with AD-like GVHD was significantly higher than that in patients with LP-like GVHD ($U = 49.000$, $P = 0.012$, Figure 3).

Expressions of loricrin and involucrin in patients with atopic dermatitis-like graft-versus-host disease and lichen planus-like graft-versus-host disease

In immunohistochemistry study, the expression level of loricrin was higher in the upper epidermis of AD-like GVHD and LP-like GVHD patients, compared with HCs [Figure 4a–4c]. The intensities of involucrin staining were also higher in lesional epidermis of patients with AD-like GVHD and LP-like GVHD, compared with HCs [Figure 4d–4f]. In Western blotting analysis, compared with HCs, the expression levels of loricrin and involucrin were also significantly increased in lesions of patients with AD-like GVHD ($t = -7.689$, $P = 0.002$ for loricrin; and $t = -22.695$, $P < 0.001$ for involucrin) and LP-like GVHD ($t = -8.979$, $P = 0.001$ for loricrin; and $t = -10.413$, $P < 0.001$ for involucrin; Figure 4g–4i).

LL37 and human β -defensins 2 expressions in patients with atopic dermatitis-like graft-versus-host disease and lichen planus-like graft-versus-host disease

The expression of LL37 and human β -defensins 2 (hBD2) in skin tissues was measured by immunohistochemistry. Compared with HCs, the expression levels of LL37 were decreased in lesional epidermis of patients with AD-like GVHD and LP-like GVHD [Figure 5a–5c]. On the other hand, the expression levels of hBD2 were higher in epidermis of patients with AD-like GVHD and LP-like GVHD [Figure 5d–5f]. Compared with HCs, the mRNA levels of LL37 were significantly decreased in lesions of AD-like GVHD ($t = 5.459$, $P = 0.005$) and LP-like GVHD patients ($t = 4.930$, $P = 0.008$; Figure 5g), while the mRNA levels of hBD2 were increased in patients with AD-like GVHD ($t = -6.842$, $P = 0.002$) and LP-like GVHD ($t = -20.391$, $P < 0.001$). Moreover, the mRNA level of hBD2 in AD-like GVHD patients was significantly higher than that in LP-like GVHD patients ($t = -10.798$, $P < 0.001$; Figure 5h).

DISCUSSION

AD-like GVHD is a novel form of cutaneous GVHD, which was first reported in 2013,^[6] and LP-like GVHD is a classical phenotype of GVHD. In this study, characteristics of patients with AD-like GVHD and LP-like GVHD were summarized for differential diagnosis. Skin manifestations of patients with AD-like GVHD and LP-like GVHD appeared at least 100 days after HSCT. Based on the time of disease onset,



Figure 2: Histopathology of HC, AD-like GVHD, and LP-like GVHD (haematoxylin and eosin staining, original magnification, $\times 400$): (a) Normal skin; (b) lesions of AD-like GVHD showed acanthosis and scattered keratinocyte necrosis in epidermis, a sparse parivascular lymphocytic infiltrate and eosinophils infiltrate in the dermis; (c) lesions of LP-like GVHD showed hyperkeratosis, focal increases in the granular cell layer, vacuolar changes of basal layer, scattered keratinocyte necrosis in the epidermis and melanophages, and a large number of lymphocytic infiltration in the dermis. HC: Healthy control, AD: Atopic dermatitis, GVHD: Graft-versus-host disease, LP: Lichen planus.

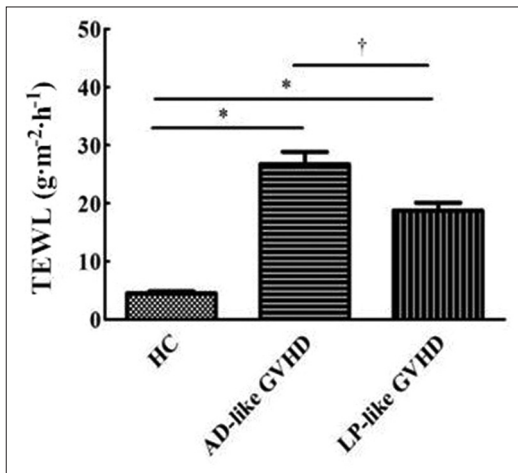


Figure 3: Transepidermal water loss in each group. Data were expressed as mean \pm SE. * $P < 0.001$, † $P < 0.05$. HC: Healthy control; AD: Atopic dermatitis; GVHD: Graft-versus-host disease; LP: Lichen planus; SE: Standard error.

patients with AD-like GVHD and LP-like GVHD were in the chronic phase of GVHD. The onset of AD-like GVHD was earlier than that of LP-like GVHD. Six out of eighteen AD-like GVHD patients received HSCT from donors with history of allergic diseases, while none of the donors for LP-like GVHD patients had history of allergic diseases. The oral mucosa and nails involvements were more common in patients with LP-like GVHD. Five out of eighteen patients with AD-like GVHD and nine out of twelve patients with LP-like GVHD had extra-skin involvements. In addition, five AD-like GVHD patients and two LP-like GVHD patients experienced onset and/or aggravation of GVHD during rapid withdrawal or taper of systemic corticosteroids or immunosuppressors.

The striking clinical features of patients with AD-like GVHD were eczematous dermatitis, pronounced itching and dry skin, increase of peripheral blood eosinophils, and/or elevated serum IgE level, while the diagnostic clinical features of LP-like GVHD are erythematous/violaceous flat-topped papules or plaques. The characteristic histopathologic changes of AD-like GVHD are epidermal spongiosis and eosinophils infiltrate, which are different from the pathological features of LP-like GVHD. In LP-like GVHD,

focal increases in the granular cell layer and vacuolar changes of basal layer often reveal in the epidermis, and melanophages and a large number of lymphocytic infiltrate are common showing in the dermis.

A number of previous studies have demonstrated that itching is a frequent phenomenon in many dermatological disorders, for example, psoriasis, eczema, AD, LP, dermatomyositis, and cutaneous lymphomas.^[12-14] Although the intensity of itching in different phenotypes of GVHD is different, no comparison has been performed so far. Based on our results, we found that the intensity of itching was much stronger in patients with AD-like GVHD than that in patients with LP-like GVHD. The itching in AD-like GVHD patients often disturbs their sleep quality. Itching induces a desire to scratch, and the scratch further disrupts permeability barrier, representing another potentially important vicious cycle in AD-like GVHD and LP-like GVHD.

The quantification of TEWL with noninvasive bioengineering method has acquired ratification as one of the most reliable methods to assess the barrier function of the skin. TEWL value is regarded as one of the most important parameters for skin barrier function.^[15] TEWL is a measure of the flux density of condensed water diffusing from the deeper highly hydrated layers of the dermis and epidermis to the skin surface. TEWL values are affected by the state and function of the stratum corneum (SC). There is convincing evidence that increased TEWL is associated with skin barrier dysfunction whereas normal or decreased TEWL is regarded as an indicator for intact or recovered skin barrier.^[16] In this research, we found that TEWL in patients with AD-like GVHD and LP-like GVHD was increased as compared with HCs, illustrating that the skin barrier function was impaired in these patients. The impaired skin barrier function induces increased TEWL that further causes dry skin and itching. The scratch further impaired skin barrier and worsen itching. In addition, we also found that TEWL in patients with AD-like GVHD was significantly higher than that in patients with LP-like GVHD. The results are consistent between TEWL values and intensity of itching.

In parallel, keratin and filaggrin create the primary structural scaffold for the cornified lipid envelope, while involucrin and

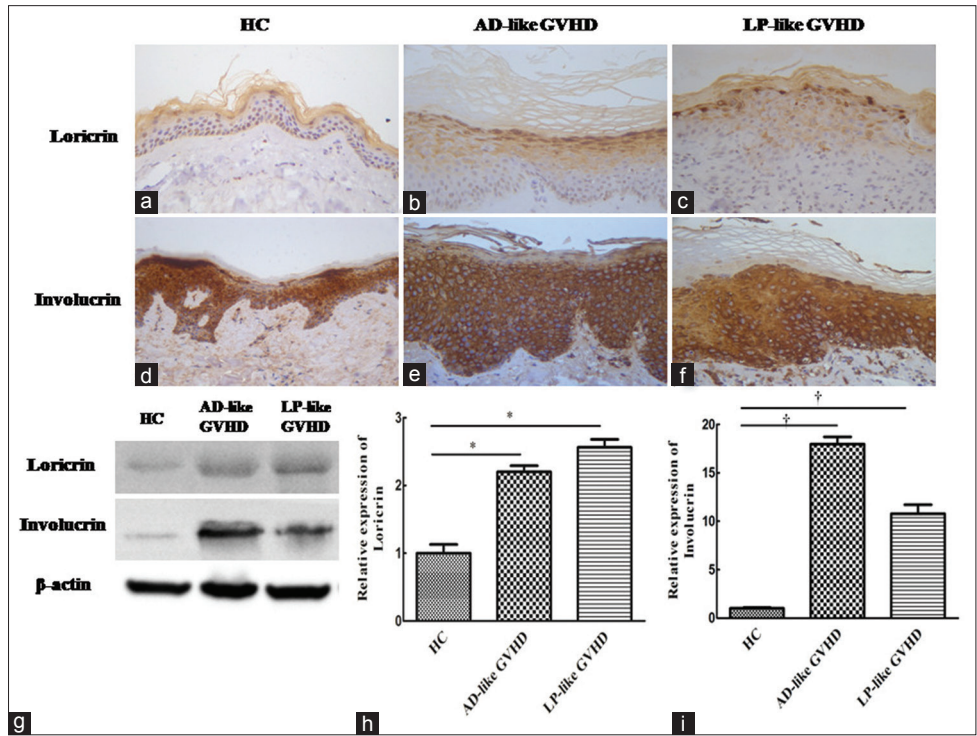


Figure 4: Immunohistochemical analysis for loricrin (a-c) and involucrin (d-f) expressions in HC and patients with AD-like GVHD and LP-like GVHD (original magnification, $\times 400$). (g-i) Loricrin and involucrin expressions by Western blotting analysis. Data were expressed as a mean \pm SE. * $P < 0.05$, † $P < 0.001$. HC: Healthy control; AD: Atopic dermatitis; GVHD: Graft-versus-host disease; LP: Lichen planus; SE: Standard error.

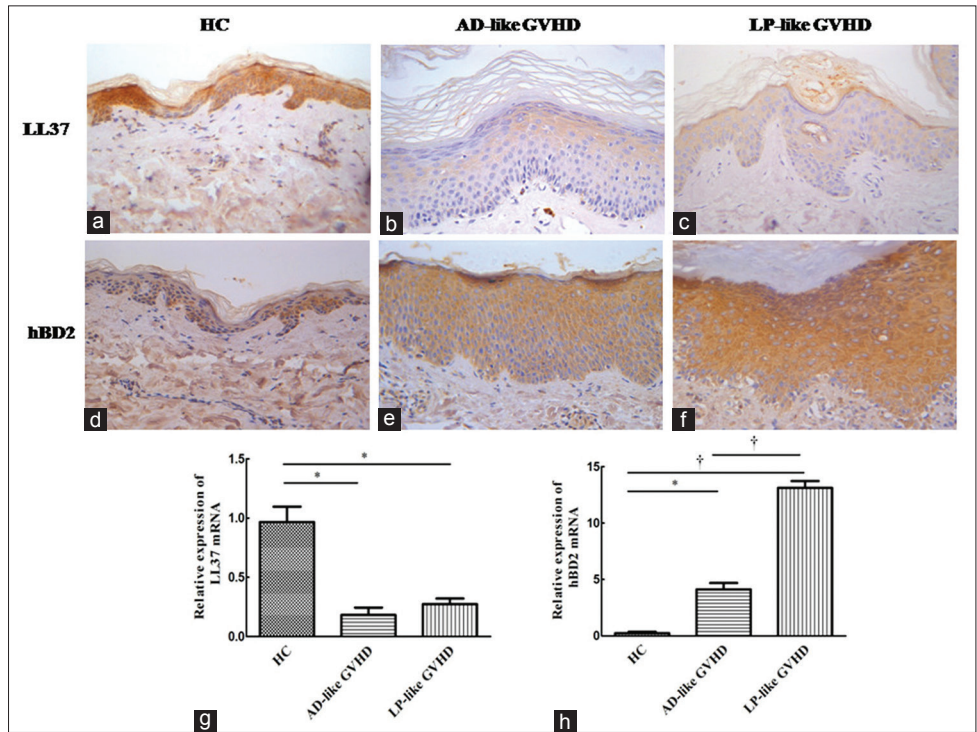


Figure 5: Immunohistochemical analysis for LL37 (a-c) and hBD2 (d-f) expressions in HC and patients with AD-like GVHD and LP-like GVHD (original magnification, $\times 400$). Relative mRNA levels of LL37 (g) and hBD2 (h). Data were expressed as a mean \pm SE. * $P < 0.05$, † $P < 0.001$. HC: Healthy control; AD: Atopic dermatitis; GVHD: Graft-versus-host disease; LP: Lichen planus; SE: Standard error; hBD2: Human β -defensins 2.

loricrin provide the filamentous framework for the binding of additional structural proteins and ceramides.^[17] Ultimately,

an effective barrier depends on the tight regulation and organization of these molecules within the SC. Although a

number of previous studies have shown that the expression of filaggrin, loricrin, and involucrin was decreased in lesions of AD,^[18-21] the expression of differentiation proteins in the epidermis of AD-like GVHD is different from AD. In this study, we revealed that expression levels of loricrin and involucrin were increased in the epidermis of AD-like GVHD and LP-like GVHD patients, compared with HCs, using both immunohistochemistry and Western blotting analysis. In our previous study, we have found that filaggrin expression was increased in lesions of patients with AD-like GVHD and LP-like GVHD. Abnormal differentiation of epidermis is existed in lesions of patients with AD-like GVHD and LP-like GVHD. These findings suggested that the mechanism of AD-like GVHD might differ from that of spontaneous AD. Overexpression of filaggrin, loricrin, and involucrin in AD-like GVHD and LP-like GVHD might be the response of skin cells to compensate abnormal function, while the low expression of these proteins was mainly due to congenital defect in spontaneous AD.

Another important function of the skin barrier is the antimicrobial function. In the skin, AMPs are secreted by keratinocytes, sebocytes, phagocytes, T cells, and mast cells with antimicrobial effects against bacterial, fungal, viruses, and parasites.^[22,23] AMPs represent one of the first evolved defense mechanisms, contributing to maintenance of the barrier function. Little is known about the change of AMPs in cutaneous GVHD. LL37 is the only one cathelicidin in humans, which is proteolytically generated by proteinase 3 in neutrophils and kallikrein 5 and 7 in epidermal keratinocytes. LL37 and hBDs exhibit a wide range of antimicrobial activity against bacteria, viruses, fungi, and parasites and control inflammation by acting as both pro- and anti-inflammatory factors. Moreover, LL37 also contributes to the maintenance of skin barrier homeostasis.^[24] LL37 is required for normal epidermal permeability barrier function and is also important for the integrity of extracutaneous epithelia.^[25] In our study, we found that LL37 expression was decreased in lesions of patients with AD-like GVHD and LP-like GVHD. In line with immunohistochemical results, LL37 mRNA expression was decreased in lesions of patients with AD-like GVHD and LP-like GVHD. The low expression of LL37 may be the response of impaired skin barrier of AD-like GVHD and LP-like GVHD. In addition, we found that hBD2 expression was increased in lesions of patients with AD-like GVHD and LP-like GVHD, and hBD2 mRNA expression was also increased in lesions of patients with AD-like GVHD and LP-like GVHD. hBD2 are commonly inducible following microbial challenge and exposure to pro-inflammatory stimuli such as lipopolysaccharide, tumor necrosis factor- α , and interleukin-1 β .^[9] When the skin barrier is impaired, a large number of microorganisms invades the skin. The overexpression of hBD2 might be the results of immunoreactions of skin barrier impairment in AD-like GVHD and LP-like GVHD.^[26]

In conclusion, the present study revealed that permeability and antimicrobial barriers are impaired in AD-like GVHD

and LP-like GVHD. These two functions are co-regulated and interdependent. The increased expression of loricrin and involucrin suggested a different mechanism of skin barrier impairment from spontaneous AD. The immunoreactions, but not the congenital defect, are considered to be the primary cause of skin barrier impairment in AD-like GVHD and LP-like GVHD.

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Conflicts of interest

There are no conflicts of interest.

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