Aberrant human tissue kallikrein levels in the stratum corneum and serum of patients with psoriasis: dependence on phenotype, severity and therapy

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Summary

Background Human tissue kallikreins (KLKs) are a family of 15 trypsin-like or chymotrypsin-like secreted serine proteases (KLK1–KLK15). Multiple KLKs have been quantitatively identified in normal stratum corneum (SC) and sweat as candidate desquamation-related proteases.

Objectives To quantify KLK5, KLK6, KLK7, KLK8, KLK10, KLK11, KLK13 and KLK14 in the SC and serum of patients with psoriasis, and their variation between lesional and nonlesional areas and with phenotype, therapy and severity. The overall SC serine protease activities were also measured.

Methods Enzyme-linked immunosorbent assays and enzymatic assays were used.

Results The lesional SC of psoriasis generally contained significantly higher levels of all KLKs. KLK6, KLK10 and KLK13 levels were significantly elevated even in the nonlesional SC. The overall trypsin-like, plasmin-like and furin-like activities were significantly elevated in the lesional SC. Plasmin-like activity was significantly elevated also in the nonlesional SC. The SC chymotrypsin-like activity was only slightly elevated in psoriasis. KLK7 serum levels did not differ between normal volunteers and patients with psoriasis. Serum KLK6, KLK8, KLK10 and KLK13 levels in patients with untreated psoriasis significantly correlated with Psoriasis Area and Severity Index score. Serum KLK5 and KLK11 levels decreased in patients with psoriasis after therapy, especially with etretinate. Patients with erythrodermic psoriasis exhibited significantly higher serum KLK levels than normal subjects or patients with psoriasis vulgaris or arthropathic psoriasis.

Conclusions We found aberrant KLK levels in the SC and serum of patients with psoriasis and suggest that KLKs might be involved in the pathogenesis of this disease.

Psoriasis is a common, chronic inflammatory skin disease. Psoriasis was recognized as a disease of disordered keratinocyte proliferation and differentiation. Currently, psoriasis is considered a T cell-mediated inflammatory disease.[1] In psoriatic lesions, T-cell and dendritic cell activation leads to nuclear factor-κB activation and release of cytokines, chemokines, proteases and other inflammatory mediators.[2] Among these well-defined molecules, which may be associated with the pathogenesis of psoriasis, and may represent potential therapeutic targets,[2] the involvement of proteases in the pathogenesis of psoriasis has not as yet been clarified.

The human tissue kallikrein (KLK) gene family localizes as a cluster to chromosome 19q13.4 and encodes 15 secreted serine proteases (KLK1–KLK15); KLK3, KLK7 and KLK9 are chymotrypsin-like enzymes, whereas the other KLKs are trypsin-like enzymes.[3] So far, at least eight different KLKs have been quantitatively identified in the stratum corneum (SC) by enzyme-linked immunosorbent assay (ELISA).[4,5] In normal skin, KLKs tend to be distributed in more highly differentiated cells, i.e. the SC, stratum granulosum and skin appendages.[6,7] KLK5, KLK7 and KLK8 are secreted into the intercellular space via lamellar granules.[8,9] KLK5 and KLK7 degrade desmosomes and/or corneodesmosomes.[10,11] According to recently accumulated evidence, KLKs are believed to be cell differentiation and/or desquamation-related serine proteases.

It has been reported that KLK2, KLK4 and KLK15 activate pro-KLK3, KLK5 activates pro-KLK7 and pro-KLK14, and pro-KLK5 is activated by autocatalysis or by KLK14, suggesting that KLKs may function as an enzymatic cascade pathway.[12,13] Protease-activated receptors (PARs) are members of transmembrane G-protein coupled receptors which are cleaved and activated by serine proteases (e.g. trypsin and thrombin). The signals are transmitted to the nucleus to mediate cell proliferation, differentiation, pain transmission and inflammatory responses.[14] KLKs and PAR-2 are colocalized in normal skin[7,15] and KLK5, KLK6 and KLK14 cleave PAR-2 at its activation site *in vitro*.[16,17] Hence, the presence of a KLK–PAR signalling pathway in the skin is conceivable. These and other data[18,19] implicate KLKs in inflammatory reactions.

In psoriasis, (mRNAs)*KLK6* and *KLK9* are increased in the skin lesion.[20] The localization of multiple (mRNAs)*KLK* and KLKs was expanded in suprabasal layers of psoriatic lesions.[7,21] Psoriatic lesions possess higher levels of active KLK7 and are characterized by elevation of chymotrypsin-like activity.[21] These collective findings suggest an involvement of KLKs in the pathogenesis of psoriasis, including aspects of keratinocyte proliferation/differentiation and inflammatory reactions.

Therefore, the present study aimed to quantitatively measure KLK5, KLK6, KLK7, KLK8, KLK10, KLK11, KLK13 and KLK14 in both the SC and serum, as well as to assess the overall serine protease activities in the SC of patients with psoriasis. The data from these patients were further compared with corresponding data obtained from normal subjects, or analysed based on the following factors: lesional skin, nonlesional skin, phenotype, therapy and severity.

Materials and methods

Sample preparation for stratum corneum analysis

Informed consent was obtained from all participants and our study was conducted according to the Declaration of Helsinki. The medical ethical committee of the Graduate School of Medical Science, School of Medicine, Kanazawa University approved all of the described studies. The SC samples from normal subjects were the same as in our previous study,[4] and were obtained from the forearms of 96 normal volunteers [30 (15 women and 15 men) in each of three age groups: 30–39, 40–49 and 50–59 years, respectively, and six (five women and one man) with ages over 70 years]. The SC samples from patients with psoriasis were obtained from the forearms of 16 patients (10 men and six women, mean ± SD age 53 ± 16 years) who had been diagnosed as having psoriasis vulgaris, showing a Psoriasis Area and Severity Index (PASI) score between 15 and 25, and treated with psoralen plus ultraviolet A (PUVA) and topical agents. Six nonlesional and lesional SC samples were provided by the same patients.

The SC samples from patients with psoriasis were further subdivided: lesional skin (n = 12), psoriatic plaques with moderate-strong infiltration; and nonlesional skin (n = 10), i.e. skin unaffected by psoriatic plaques. Tape stripping was performed using Nichiban[TM] tape (organic solvent-stable tape with organic solvent-soluble adhesive; Nichiban, Tokyo, Japan). The SC samples were washed and purified using toluene. After toluene treatment, the purified samples were air-dried and weighed. The detailed procedure is described elsewhere.[4]

Sample preparation for serum analysis

Serum samples were obtained from 90 healthy volunteers (a mixture of men and women, age range 21–50 years). Serum samples from patients with psoriasis were obtained from 96 subjects (mean ± SD age 50 ± 16 years) who were diagnosed as having psoriasis vulgaris (n = 76), arthropathic psoriasis (n = 13) and erythrodermic psoriasis (n = 7). The severity of skin lesions was evaluated using PASI for each patient. The patients were also subdivided by types of therapy, i.e. no therapy, ciclosporin, etretinate, PUVA, corticosteroid topical agents, and vitamin D_3 topical agents. When a patient received a combination of the therapies, the main therapy was suggested following a priority rule: (i) oral therapies, (ii) PUVA therapy, and (iii) therapy with any topical agent.

Immunofluorometric enzyme-linked immunosorbent assays for human tissue kallikreins

With the exception of Fuso-FB6MA53 anti-KLK11 antibody (Ab), which was purchased (Fuso, Osaka, Japan), all other monoclonal and polyclonal anti-KLK Abs were developed in our laboratory.[7] Each of the Abs displayed negligible cross-reactivity with other KLKs (data not shown). The detailed procedure and conditions for sample preparation and each KLK ELISA assay are described elsewhere.[4]

Assay of serine protease enzymatic activities in the stratum corneum

The synthetic peptide substrates Boc-Phe-Ser-Arg-AMC (FSR), Boc-Pro-Phe-Arg-AMC (PFR), Pyr-Arg-Thr-Lys-Arg-AMC (R-KR) and Boc-Val-Leu-Lys-AMC (VLK) (Bachem, Torrance, CA, U.S.A.) for the trypsin-like activities were used at 0·1 mmol L^{-1} final concentration. MeO-Suc-Arg-Pro-Tyr-pNA-HCl (RPY) (Chromogenix, Milan, Italy) for the chymotrypsin-like activity was used at 0·4 mmol L^{-1} final concentration.

The reaction mixtures for the SC samples consisted of 0·5 mg dry weight of the SC, 10 μL of N,N-dimethylformamide, 240 μL of 0·1% Triton X-100, 175 μL of 0·2 mol L^{-1} Tris-HCl buffer (pH 8·0) and 50 μL of substrates.[4] They were incubated at 37 °C with shaking for 2 or 4 h. Released 7-amino-4-methyl-coumarin (AMC) was measured using a fluorescence spectrophotometer (Wallac Victor 2 1420 Multilabel Counter; Perkin Elmer, Boston, MA, U.S.A.) and released *para*-nitroanilide (pNA) using the same instrument at 405 nm. Porcine trypsin type II (trypsin tablets; Sigma, St Louis, MO, U.S.A.; molecular weight 23·8 kDa) and plasmin (Sigma) were used as positive controls. Each assay was performed in triplicate.

Statistical analysis

Bartlett's test was performed first to determine the equality of variances among the specified groups. If significant differences were found with Bartlett's test, then the Kruskal–Wallis test was performed. Reported P-values were adjusted by Dunn's method to reflect multiple comparisons. When a significant difference was not found with Bartlett's test, both the Kruskal–Wallis test and one-way ANOVA were performed. When comparing two groups, the Mann–Whitney test or the Wilcoxon matched pairs test was used. All statistical tests were performed using GraphPad Prism 4 version 4.02 software (GraphPad Software, Inc., San Diego, CA, U.S.A.).

Results

Kallikrein levels in the stratum corneum of normal subjects and patients with psoriasis (nonlesional and lesional areas)

We used ELISAs developed in-house to quantify KLK5, KLK6, KLK7, KLK8, KLK10, KLK11, KLK13 and KLK14 in SC extracts (ng mg^{-1} dry weight) (Fig. 1). The SC samples of the normal subjects (n = 96) were the same as in our previous study.[4] The psoriasis SC samples were obtained from patients diagnosed as having psoriasis vulgaris. These were subdivided as nonlesional (n = 10), indicating that the skin was not affected by psoriatic plaques; and lesional (n = 12), which included chronic psoriatic plaques with infiltration. In our figures, dots connected with a line represent samples acquired from both lesional and nonlesional areas in six patients. Paired comparisons were performed for the six patients who provided both the nonlesional and lesional SC samples. A significant difference (P < 0·05) was observed for all studied KLKs, not only by paired t-test but also by Wilcoxon matched pairs test in these patients. KLK levels in lesional skin were higher than in nonlesional skin in all six patients (Fig. 1).

When differences among the groups of normal, nonlesional and lesional samples were examined, the use of Bartlett's test rejected the equality of variances among these groups. Then, the Kruskal–Wallis test with the *post hoc* test (Dunn's method) was performed to reflect multiple comparisons and showed significant differences (P < 0·05 for each) (Fig. 1). These significant differences are represented by * (lesional vs. nonlesional and normal skin) and # (nonlesional vs. normal skin). In short, the differences between lesional vs. nonlesional and normal skin were significant for all KLKs tested.

The mean values of KLK5, KLK7, KLK8, KLK10, KLK11 and KLK14 in lesional vs. nonlesional SC were two- to five-fold higher (Fig. 1a–f), with the exception of one case in which the level of KLK5 was equivalent (*c.* 12 ng mg^{-1}) between the nonlesional and lesional SC (Fig. 1d). The mean values of
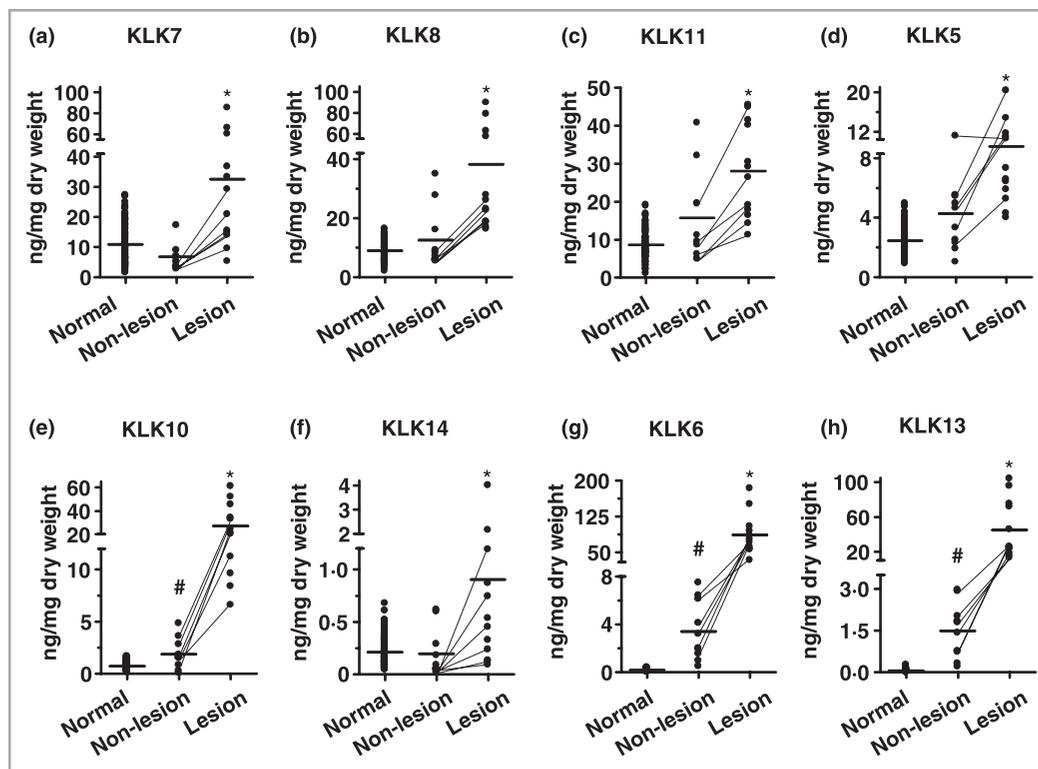
Fig 1. Differences in kallikrein (KLK) concentrations in the stratum corneum (SC) of normal subjects and patients with psoriasis. KLK levels in the SC are shown (ng mg^{-1} dry weight). As the median and mean of each group were almost identical in all KLKs, only the mean (horizontal bars) is shown. The connected dots indicate paired samples (from the same patients). Kruskal–Wallis test with the *post hoc* test (Dunn's method) identified significant differences (P < 0·05 for each) as represented by: *, lesional vs. nonlesional and normal skin; #, nonlesional vs. normal skin.

KLK6 and KLK13 in lesional SC were 20- to 30-fold higher (86 and 45 ng mg^{-1}, respectively) compared with those in nonlesional SC (3·4 and 1·5 ng mg^{-1}, respectively) (Fig. 1g,h). In the nonlesional samples, the amounts of KLK7 and KLK14 were within the normal range (Fig. 1a,f). KLK5, KLK8 and KLK11 also remained within the normal range in most nonlesional samples, with a few exceptions which displayed higher values than the normal subjects (Fig. 1b,c). The amounts of KLK6, KLK10 and KLK13 in the nonlesional skin (0·1–7·5 ng mg^{-1}) were significantly higher than in normal subjects (< 1·7 ng mg^{-1}, P < 0·05 for each) (Fig. 1e,g,h).

Stratum corneum serine protease enzymatic activities in normal subjects and patients with psoriasis

The overall SC enzymatic activities were measured in SC samples from normal volunteers and patients with psoriasis (Table 1). In this study, 'trypsin-like activities' refer to the activities of enzymes towards Boc-Phe-Ser-Arg-AMC (FSR) and Boc-Pro-Phe-Arg-AMC (PFR) fluorogenic substrates. 'Chymotrypsin-like activity', 'plasmin-like activity' and 'furin-like activity' refer to the activities of enzymes towards MeO-Suc-Arg-Pro-Tyr-pNA-HCl (RPY), Boc-Val-Leu-Lys-AMC (VLK) and Pyr-Arg-Thr-Lys-Arg-AMC (R-KR) substrates, respectively. Due to the limited amount of SC from these individuals, the samples used for each substrate were randomly chosen from the subjects indicated in Fig. 1. The number of subjects chosen was the same (n = 12 for the normal; n = 6 for the nonlesional and lesional samples), although the individuals were not always the same. When the SC samples were boiled for 5 min, the enzymatic activity was completely lost (data not shown).

The trypsin-like activity towards FSR substrate in the nonlesional samples (13·5 nmol mg^{-1} dry weight) did not differ significantly from that in normal SC (12·1 nmol mg^{-1}) but the activity in lesional samples (62·2 nmol mg^{-1}) was signifi-

cantly higher than that in normal and nonlesional skin (P < 0·05 for each comparison) (Table 1). The trypsin-like activity towards PFR substrate and the chymotrypsin-like activity towards RPY substrate were moderately elevated in the nonlesional and lesional samples but no statistically significant differences were noted (Table 1). The plasmin-like activity towards VLK substrate was significantly elevated in both nonlesional and lesional samples (5·8 and 14·5 nmol mg^{-1}, respectively, P < 0·05 for each) in comparison with normal skin (1·7 nmol mg^{-1}) (Table 1). The furin-like activity towards R-KR substrate in nonlesional samples (8·5 nmol mg^{-1}) was higher than in normal skin (3·0 nmol mg^{-1}) but was not significantly different (P > 0·05). The activity in lesional SC was increased significantly, by approximately eightfold (26·2 nmol mg^{-1}), in comparison with normal skin (P < 0·05) (Table 1).

Kallikrein levels in serum of normal subjects and patients with psoriasis of various phenotypes

The concentrations of KLK5, KLK6, KLK7, KLK8, KLK10, KLK11, KLK13 and KLK14 in serum (ng mL^{-1}) were quantified for normal and psoriasis subjects and the results were analysed according to phenotypes, severity and therapies (Table 2, Figs 2, 3). As these KLKs have mainly either chymotrypsin-like (KLK7) or trypsin-like activity (KLK5, KLK6, KLK8, KLK10, KLK11, KLK13 and KLK14),[3] they have also been grouped as 'chymotrypsin-like KLK' and 'trypsin-like KLKs'. In Table 2, the patients with psoriasis were subdivided into those with psoriasis vulgaris (n = 59), arthropathic psoriasis (n = 12) and erythrodermic psoriasis (n = 7). Data shown in Table 2 are from patients who have been treated (the treatment modalities were not classified); patients without therapy were excluded due to their small number (n = 18).

The levels of KLK5 and KLK11 were decreased in psoriasis vulgaris and arthropathic psoriasis compared with normal

Table 1 Stratum corneum serine protease enzymatic activities (mean ± SD) in normal subjects and patients with psoriasis

Substrate Released AMC or pNA (nmol mg^{-1} dry weight)	Normal skin (n = 12)	Psoriasis	
		Nonlesional skin (n = 6)	Lesional skin (n = 6)
Trypsin-like activity			
Phe-Ser-Arg-AMC	12·1 ± 4·1	13·5 ± 5·4	62·2 ± 15·7*
Pro-Phe-Arg-AMC	5·6 ± 2·7	6·2 ± 2·4	11·0 ± 1·9
Chymotrypsin-like activity			
Arg-Pro-Tyr-pNA	11·1 ± 5·3	15·3 ± 7·7	15·8 ± 6·7
Plasmin-like activity			
Val-Leu-Lys-AMC	1·7 ± 1·0	5·8 ± 2·4#	14·5 ± 5·5**
Furin-like activity			
Pyr-Arg-Thr-Lys-Arg-AMC	3·0 ± 1·3	8·5 ± 4·4	26·2 ± 9·5*

The overall stratum corneum serine protease enzymatic activities represent released AMC or pNA from the synthetic substrates (nmol mg^{-1} dry weight). The amounts of released AMC or pNA were measured at 2 h or 4 h, respectively. The *post hoc* test (Dunn's method) identified significant differences (P < 0·05 for each) for the following: *, lesional vs. nonlesional and normal skin; **, lesional vs. normal skin; #, nonlesional vs. normal skin. AMC, 7-amino-4-methylcoumarin; pNA, *para*-nitroanilide.

Table 2 Kallikrein (KLK) concentrations in serum of normal subjects and patients with psoriasis, measured by enzyme-linked immunosorbent assay

KLK (ng mL^{-1})	Normal (n = 90)	Psoriasis vulgaris (n = 59)	Arthropathic psoriasis (n = 12)	Erythrodermic psoriasis (n = 7)
PASI		11·5 ± 5·6	10·7 ± 5·9	34·9 ± 6·8#a
Chymotrypsin-like KLK				
KLK7	5·1 ± 2·1	4·6 ± 2·6	5·6 ± 2·1	7·1 ± 2·6#b
Trypsin-like KLKs				
KLK6	4·4 ± 1·5	5·3 ± 2·8	4·9 ± 1·7	16·4 ± 7·1#c
KLK8	1·9 ± 0·77	2·1 ± 1·3	2·1 ± 1·1	8·2 ± 4·1#d
KLK10	1·2 ± 0·56	1·3 ± 0·62	1·4 ± 0·70	3·4 ± 3·0#c
KLK5	0·68 ± 0·15	0·51 ± 0·22*	0·55 ± 0·30*	0·90 ± 0·37#a
KLK11	0·55 ± 0·16	0·28 ± 0·14*	0·26 ± 0·17*	0·61 ± 0·25#a
KLK14	0·23 ± 0·095	0·31 ± 0·28	0·41 ± 0·37	0·49 ± 0·37#c
KLK13	0·01	0·078 ± 0·051	0·11 ± 0·085	0·27 ± 0·21#b

The values for KLKs and Psoriasis Area and Severity Index (PASI) are given as mean ± SD. Data are shown for treated patients only. As KLK13 was not always detectable in normal subjects, KLK13 in normal subjects is shown as 0·01 ng mL^{-1} (the minimum detection value) and the data were excluded from the statistics. The *post hoc* test (Dunn's method) identified significant differences (P < 0·05 for each) as follows: *, the specified phenotype vs. normal; #a, erythrodermic psoriasis vs. psoriasis vulgaris and arthropathic psoriasis; #b, erythrodermic psoriasis vs. psoriasis vulgaris; #c, erythrodermic psoriasis vs. normal; #d, erythrodermic psoriasis vs. psoriasis vulgaris and normal.

subjects (P < 0·05 for each comparison) (Table 2). Otherwise, no significant differences in KLK levels were detected between normal subjects and those with psoriasis vulgaris and arthropathic psoriasis. However, in erythrodermic psoriasis all KLKs were elevated in comparison with all other groups (P < 0·05 for each, see the details in Table 2).

As the group with erythrodermic psoriasis showed significant differences, in comparison with normal subjects or other phenotypes of psoriasis, erythrodermic psoriasis was excluded from the following analysis.

Kallikrein levels in the serum of patients with psoriasis with or without therapy

KLK concentrations in serum are reported in the groups of normal subjects (n = 90), patients with psoriasis without therapy [therapy (−), n = 18] and patients with psoriasis undergoing therapy [therapy (+), n = 71] (Fig. 2). The psoriasis group includes psoriasis vulgaris and arthropathic psoriasis, as no significant differences were detected between these groups (Table 2). The therapy (+) patients were under treatment with any combination of oral and topical agents and/or PUVA.

For KLK7, we found no significant differences among the normal, therapy (+) or therapy (−) patients (Fig. 2a, left) and no significant correlations between KLK7 and PASI (Fig. 2a, right). Similarly, the distributions of KLK6 and KLK8 did not differ significantly among the normal, therapy (+) or therapy (−) groups (Fig. 2b,c, left). The therapy (−) group demonstrated a significant correlation between KLK6 or KLK8 levels and PASI (r = 0·70 and r = 0·61, respectively, P < 0·05 for each comparison). The correlation was not apparent in the therapy (+) group (Fig. 2b,c, right). For KLK10, the therapy

(−) group displayed a significantly higher concentration than the normal group (P < 0·05) (Fig. 2d). KLK10 levels in the therapy (−) group correlated significantly with PASI (r = 0·51, P < 0·05), but this was not seen in the therapy (+) group (Fig. 2d). The distribution of KLK5 in the therapy (+) group was significantly lower compared with normal subjects (P < 0·05) (Fig. 2e). The distributions of KLK11 in the therapy (−) and therapy (+) groups were significantly lower compared with the normal group (P < 0·05 for each) (Fig. 2f). No significant correlation was detected between PASI and KLK5 or KLK11 levels in the therapy (−) and therapy (+) groups (Fig. 2e,f). KLK14 levels in several therapy (+) patients were above the normal range (Fig. 2g). A significant correlation between KLK14 levels and PASI was not observed (Fig. 2g). The KLK13 distribution in the therapy (−) group was significantly higher compared with that in the therapy (+) group (Fig. 2h). In the therapy (−) group, there was a significant correlation between KLK13 levels and PASI (r = 0·50, P < 0·05) (Fig. 2h).

Kallikrein levels in the serum of patients with psoriasis among therapy groups

KLK concentrations in serum were analysed according to the type of therapy of patients with psoriasis (Fig. 3). Patients with psoriasis included those with psoriasis vulgaris and arthropathic psoriasis. In total, we included 81 patients with psoriasis and 90 normal subjects. The patients with psoriasis were subdivided according to the type of treatment: no therapy [therapy (−), n = 18]; ciclosporin (n = 7, treated with ciclosporin plus any topical agents); etretinate (n = 7, treated with etretinate plus any topical agents); steroid (n = 34, corticosteroid topical agent only); vitamin D$_3$ (n = 8,
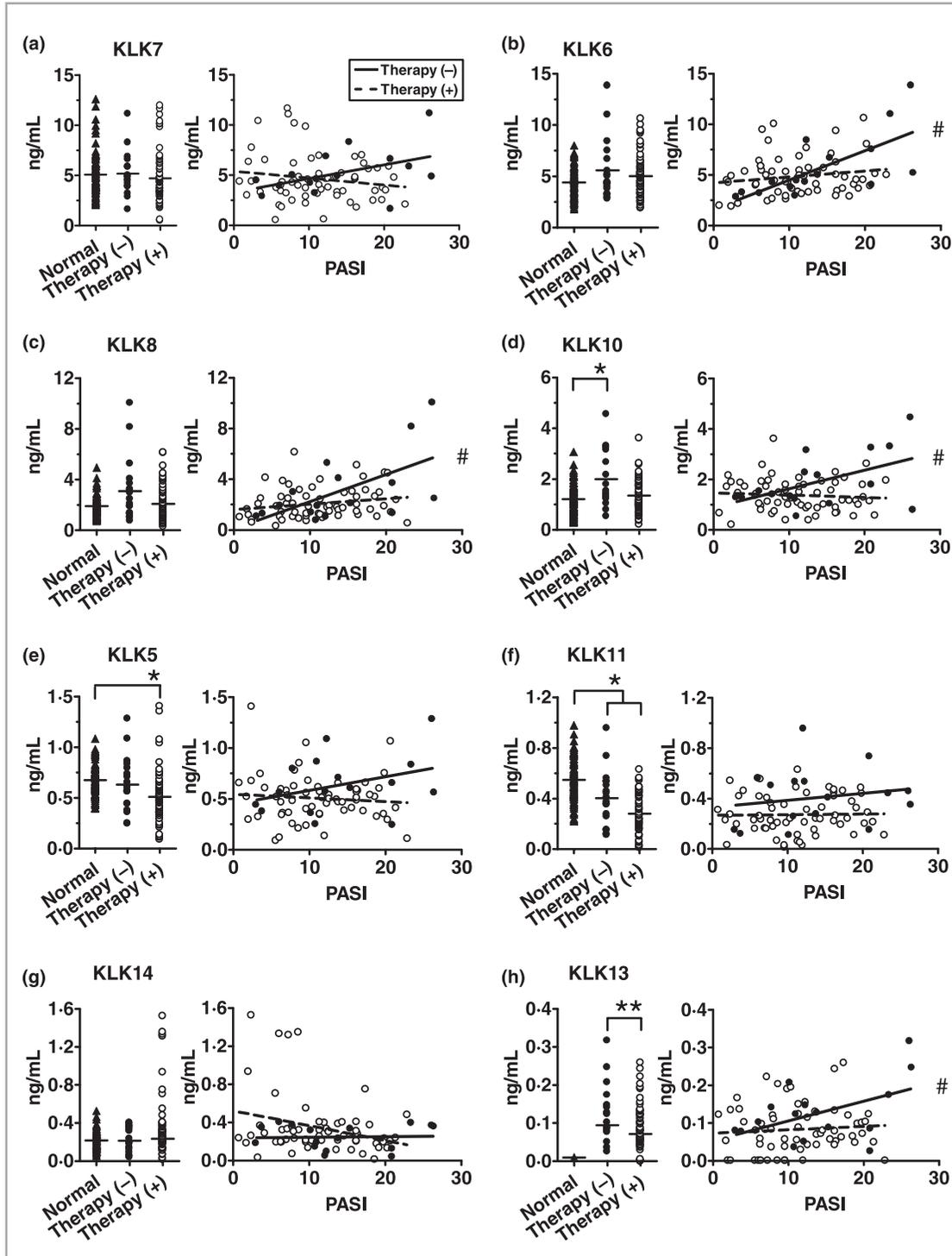
Fig 2. Kallikrein (KLK) concentrations in serum of patients with psoriasis with or without therapy. Horizontal bars indicate means. Patients include those with psoriasis vulgaris and arthropathic psoriasis. The subjects were further subdivided into normal (closed triangles, n = 90), therapy (−) (closed circles, n = 18) and therapy (+) (open circles, n = 71) (for more details see text). The left panels present the distribution of KLK values, which were analysed by Kruskal–Wallis test with the *post hoc* tests, Dunn's multiple comparison test (a–g) or Mann–Whitney test (h). In the right panels, data from patients with psoriasis were also used for regression analysis between Psoriasis Area and Severity Index (PASI) and KLK levels. As KLK13 was not always detectable in normal subjects, KLK13 levels in normal subjects are shown as a dot at 0.01 ng mL^{-1} (the minimum detection value) and are excluded from the statistics (h). *, significant differences (P < 0·05) between the specified groups by Dunn's multiple comparison test; **, significant differences (P < 0·05) between the specified groups by Mann–Whitney test; #, the slopes were significantly nonzero (P < 0·05). The equations of the regression lines are: KLK6, $y = 0.29x + 1.6$; KLK8, $y = 0.21x + 0.079$; KLK10, $y = 0.073x + 0.90$; and KLK13, $y = 0.0052x + 0.054$.
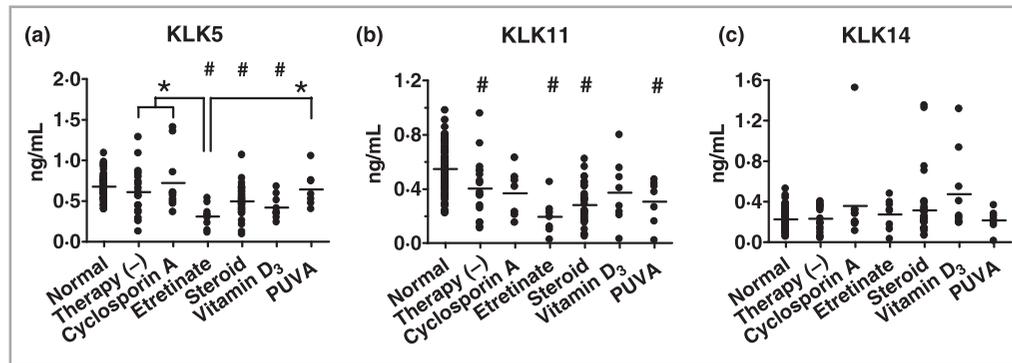
Fig 3. Differences of kallikrein (KLK) concentrations in serum of patients with psoriasis undergoing therapy. Serum concentrations of KLK5, KLK11 and KLK14 are shown (ng mL^{-1}). The data for the remaining KLKs are not shown due to the absence of significant differences. Horizontal lines indicate means. Patients include those with psoriasis vulgaris and arthropathic psoriasis. The patients were subdivided by the type of treatment: no therapy [therapy (−), n = 18], ciclosporin (n = 7), etretinate (n = 7), psoralen plus ultraviolet A (PUVA, n = 7), steroid (n = 34) and vitamin D$_3$ (n = 8) (for details see text). Kruskal–Wallis test with the *post hoc* test (Dunn's method) identified significant differences (P < 0·05 for each) as follows: #, normal vs. the specified group; *, etretinate vs. the specified group.

vitamin D$_3$ topical agent only, or vitamin D$_3$ plus corticosteroid topical agents); PUVA (n = 7, PUVA plus any topical agents).

The groups of etretinate, steroid and vitamin D$_3$ displayed significantly lower KLK5 distributions in comparison with the normal subjects (P < 0·05 for each comparison) (Fig. 3a). Furthermore, KLK5 levels in the etretinate group were significantly lower than those in the therapy (−), ciclosporin and PUVA groups (P < 0·05 for each) (Fig. 3a). In the therapy (−), etretinate, steroid and PUVA groups, the levels of KLK11 were significantly lower compared with normal subjects (Fig. 3b). No significant differences were detected in KLK14 among all therapy groups (Fig. 3c). However, several psoriasis subjects in the ciclosporin, steroid or vitamin D$_3$ groups showed a high KLK14 level (> 0·5 ng mL^{-1}) (Fig. 3c). The levels of KLK6, KLK7, KLK8, KLK10 and KLK13 did not differ significantly in the same analysis (data not shown).

Discussion

The present study aimed to quantify the tissue KLKs KLK5, KLK6, KLK7, KLK8, KLK10, KLK11, KLK13 and KLK14 in the SC and serum, as well as the overall serine protease activities in the SC of patients with psoriasis. These data were compared with those from normal subjects or analysed based on various factors such as lesional SC, nonlesional SC, phenotype, therapy and severity.

KLKs contribute to the overall SC trypsin-like (FSR) and chymotrypsin-like (RPY) activities, which are known as desquamation-related SC protease activities.[4,10,11,22] The pro-lympho-epithelial Kazal-type-related inhibitor (LEKTI) is believed to be a negative regulator of desquamation-related proteases including KLKs.[23–28] The furin-like activity of SC can proteolytically process pro-LEKTI at (R-KR↓) to 15 individual bioactive domains.[25,29,30]

LEKTI possesses an efficient inhibitory function towards the overall FSR and RPY activities in the SC[26,28] (also our unpub-

lished data). The elevation of furin-like (R-KR) activity, observed in the SC of psoriasis, could efficiently process pro-LEKTI, which is expressed in the skin lesions of psoriasis,[31] to release increased amounts of LEKTI active inhibitory domains.

KLK7 is believed to represent the major player of the chymotrypsin-like activity in normal skin.[4,32] KLK7 proform is converted to the active form in the psoriatic scales, and the chymotrypsin-like activity correlates with the amount of active KLK.[21] However, in our study, despite the significantly different amounts of immunoreactive KLK7 between the nonlesional and lesional SC of psoriasis (Fig. 1), the RPY activity was similar between these groups (Table 1). It seems that there is a discrepancy between the previously published and our present data. Considering the preference of LEKTI inhibition towards RPY activity[26,28] (also our unpublished data), the similar SC RPY activity among normal skin and psoriasis nonlesional and lesional skin could be explained by an efficient inhibitory function by LEKTI domains.

According to their kinetic properties, KLK5, KLK6, KLK8, KLK13 and KLK14 strongly display trypsin-like (FSR) activity.[33–37] The elevation of FSR activity in the SC of psoriasis lesions was more prominent (Table 1) compared with that of KLK7. Hence, FSR activity by trypsin-like KLKs might overcome LEKTI inhibition. The enzymatic activities towards FSR and PFR substrates are both classified as 'trypsin-like' activity. However, the magnitude of elevation in the lesional SC of psoriasis was dissimilar between the substrates, suggesting involvement of enzymes with distinct specificities.

As LEKTI also possesses anti-plasmin function,[25] we measured overall SC 'plasmin-like (VLK) activity' in the SC (Table 1). However, the elevated VLK activity in the lesions of patients suggests that LEKTI may not be an efficient inhibitor for the overall SC 'plasmin-like activity'. In addition, the plasmin-like activity was significantly elevated even in the nonlesional SC, in addition to the lesional SC of psoriasis (Table 1), suggesting that this activity could be involved in a preinflammatory and/or inflammatory reaction in the SC of psoriasis.

The levels of chymotrypsin-like KLK (KLK7) in the serum did not differ significantly among normal subjects and patients with psoriasis without and with therapy. Our data suggest that chymotrypsin-like KLK in serum and the overall SC chymotrypsin-like activity are unlikely to be significantly affected in psoriasis.

In normal SC, KLK6, KLK10, KLK13 and KLK14 are found at relatively low levels.[4] However, these KLKs are significantly increased in the lesional SC of psoriasis (Fig. 1). KLK6, KLK10 and KLK13 levels were significantly elevated even in the non-lesional SC (Fig. 1), suggesting that their expression could affect the emergence of the psoriatic lesion, e.g. the Köbner phenomenon and/or preinflammatory and inflammatory reactions in nonlesional skin. KLK6, KLK8, KLK10 and KLK13 levels in serum were significantly correlated with PASI in therapy (−), but not in therapy (+) patients (Fig. 2). Taken together with the elevation of KLK6, KLK10 and KLK13 in the non-lesional SC, these data suggest that these KLKs could be therapeutic targets for this disease.

KLK5 and KLK11 levels were significantly lower in the serum of therapy (+) patients (Fig. 2), suggesting that their expression could be suppressed by therapies. In particular, etretinate might selectively affect KLK5 and KLK11 levels in serum (Fig. 3).

The distributions of KLK14 and KLK7 levels in the SC of psoriasis were similar; these KLKs in the nonlesional SC are within the normal range, while in half of the subjects the lesional SC showed higher KLK7 or KLK14 values than the normal subjects (Fig. 1). KLK14 possesses both trypsin-like and chymotrypsin-like kinetic properties towards synthetic substrates,[37] whereas KLK14 tends to act as a chymotrypsin-like enzyme towards physiological substrates.[38,39] The tendency of KLK14 to behave as a chymotrypsin-like enzyme could explain the similarity with KLK7. As high levels of KLK14 in serum were observed only in the therapy (+) group (Fig. 2), KLK14 expression in serum could be affected by therapies.

The distribution of KLK levels in ciclosporin and therapy (−) groups tended to be similar (data not shown for KLK6, KLK7, KLK8, KLK10 and KLK13), implying that ciclosporin might not affect KLK expression.

In serum, no significant difference was found in KLK levels between psoriasis vulgaris and arthropathic psoriasis (Table 2). Therefore, KLKs are unlikely to be distinguishing factors for these phenotypes. The patients with erythrodermic psoriasis showed significant elevations of all KLKs in serum compared with normal, psoriasis vulgaris or arthropathic psoriasis (Table 2). The regulation of KLKs in erythrodermic psoriasis may thus be different from the other psoriatic forms.

KLKs and PAR-2 are colocalized not only in normal skin, but also in psoriatic lesions, where their expression extends into the suprabasal layers.[7,15,40] Together with the high levels of KLKs in the lesional SC, the increased KLK levels could contribute to an accelerated cell proliferation and/or inflammatory responses via PARs. Neutrophil migration, e.g. Munro's microabscess, into the skin lesion is one of the most specific features of psoriasis. Accumulating evidence indicates that interleukin (IL)-8

can mediate neutrophil migration/accumulation in skin tissue and in psoriatic lesions.[41–43] Human airway trypsin-like protease, extensively expressed in the epidermis of psoriatic lesions, released IL-8 via PAR-2 signalling.[40] Similarly, KLKs could play a role in IL-8 release and/or neutrophil migration via PARs. PS-519, a synthetic proteasome inhibitor affecting the chymotrypsin-like activity of the 20s proteasome with limited activity for serine proteases, reduced superantigen-mediated T-cell activation.[44] In addition, PS-519 proved to be therapeutically effective in a xenogenic psoriasis transplantation model;[44] hence KLKs could also be associated with T-cell activation.

The KLK–kinin system is an endogenous metabolic cascade, catalysing the release of vasoactive kinins (bradykinin-related peptides).[45] KLK1 cleaves low-molecular-weight kininogen to produce kinin peptide, which binds to kinin receptors and triggers a wide spectrum of biological effects, involving apoptosis, inflammation, proliferation, angiogenesis and neurogenesis in different animal models.[46,47] It is possible that some other KLKs might be involved in the KLK–kinin system as well. Further studies are necessary to elucidate the point.

In summary, we report for the first time the quantification of multiple KLKs in the SC and serum of patients with psoriasis. Our data suggest that KLKs, along with other proteolytic enzymes, might be involved in the pathogenesis of psoriasis. The finding of aberrant KLK levels might help in the understanding of the mechanisms of abnormal keratinization and systemic inflammatory reactions commonly seen in psoriasis. Moreover, KLK levels seem to be modified by some therapies, and KLKs could be candidate therapeutic targets in psoriasis.

Acknowledgments

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