

Aberrant Serine Protease Activities in Atopic Dermatitis

Shin Morizane MD, PhD, ^{a*}, Ko Sunagawa MD, ^a, Hayato Nomura MD, PhD, ^a,

Mamoru Ouchida PhD ^b

^a Department of Dermatology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Japan.

^b Department of Molecular Oncology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Japan.

*Correspondence author at: Department of Dermatology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1 Shikata-cho, Kita-ku, Okayama, 700-8558, Japan.

E-mail: zanemori@cc.okayama-u.ac.jp

Text word count:2,048, number of reference:52, tables:0, figures:2

Abbreviations

Atopic dermatitis (AD); kallikrein-related peptidases (KLKs); lymphoepithelial Kazal-type-related inhibitor (LEKTI); protease-activated receptor 2 (PAR2); Netherton syndrome (NS)

Abstract

Atopic dermatitis (AD) is a chronic inflammatory skin disease; the three major factors responsible for AD, i.e., epidermal barrier dysfunction, allergic inflammation, and itching, interact with each other to form a pathological condition. Excessive protease activities are characteristic abnormalities that affect the epidermal barrier in patients with AD. In normal skin, epidermal serine protease activities are controlled by kallikrein-related peptidases (KLKs) and their inhibitors, including lympho-epithelial Kazal-type-related inhibitor (LEKTI). In AD lesions, KLKs are excessively expressed, which results in the enhancement of epidermal serine protease activities and facilitates the invasion by allergens and microorganisms. In addition, some KLKs can activate protease-activated receptor 2 (PAR2) in epidermal keratinocytes and peripheral nerves, resulting in the induction of inflammation and itching. Furthermore, in AD patients with single nucleotide polymorphism (SNP) such as E420K and D386N of *SPINK5* which encodes LEKTI, LEKTI function is attenuated, resulting in the activation of KLKs and easy invasion by allergens and microorganisms. Further analysis is needed to elucidate the detailed mechanism underlying the control of serine protease activities, which may lead to the development of new therapeutic and prophylactic agents for AD.

Keywords

Atopic dermatitis, serine protease, kallikrein-related peptidases, lympho-epithelial Kazal-type-related inhibitor, protease-activated receptor 2

Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin disease; the three major factors responsible for AD, i.e., epidermal barrier dysfunction, allergic inflammation, and itching, interact with each other to form a pathological condition [1].

The most well-known factor causing epidermal barrier dysfunction is a mutation in the filaggrin gene. For the first time in 2006, it became clear that filaggrin gene mutations are an important onset-related factor, but the positive rate of filaggrin gene mutations in AD patients is not always high, and not all individuals with filaggrin gene mutations have AD [2, 3]. Therefore, it is thought that there are other causes of barrier dysfunction other than filaggrin.

Excessive serine protease activity is another characteristic abnormality that affects the epidermal barrier in AD patients. Its importance in the skin has long been noted. For example, in 1969, Song et al. reported that a trypsin-like protease is generated by skin cells [4]. In 1970, Rajka suggested that trypsin might be an important mediator of pruritus in the skin. [5]

Voegeli et al. reported increased stratum corneum serine protease activities in acute AD lesions [6]. Contrastingly, in our previous study, the serine protease activities in the strata cornea of the forearms of patients with AD were significantly enhanced not only in the lesions but also in the non-lesions [7]. In addition, these activities were significantly correlated with several biomarkers of AD [7]. Furthermore, when the epidermal serine protease activities were measured over time in two

patients treated with dupilumab, results showed that the activities decreased and normalized, almost in agreement with the Eczema Area and Severity Index score [8]. Epidermal serine protease activities are mainly controlled by kallikrein-related peptidases (KLKs) and their inhibitors such as lympho-epithelial Kazal-type-related inhibitor (LEKTI).

Epidermal keratinocyte-producing kallikrein-related peptidases

In 1930, a protease capable of releasing bradykinin from kininogen was reported, which was named "kallikrein" (derived from the Greek word "kallikreas," which means "pancreas") [9]. This protease is known as tissue kallikrein 1 (KLK1). Later, plasma kallikrein (KLKB1), which is primarily produced in the liver, was discovered as another kallikrein circulating in the blood [10]. Currently, 15 types of tissue kallikreins are known, and the 14 kallikreins, except KLK1, are called kallikrein-related peptidases (KLK2-KLK15) [11]. *KLK1-KLK15* form a gene cluster on the long arm of chromosome 19 (*KLKB1* is located on chromosome 4), and *KLK1* to *KLK15* are transcribed to mRNA with five coding exons [11]. KLKs are produced as pre-proproteins with signal peptides at the N-termini [11]. The signal peptide is removed during extracellular secretion, and the propeptide is removed extracellularly via autocatalysis or other KLKs or endopeptidases [11].

KLK5 (trypsin-like serine protease) and KLK7 (chymotrypsin-like serine protease) are serine proteases produced by cutaneous epidermal keratinocytes. These KLKs target cell adhesion

molecules, such as desmoglein-1, DSG1 (desmoglein-1, DSG1), desmocollin-1 (DSC1), and corneodesmosin (CDSN), as substrates in the stratum corneum of normal skin [12]. This process is thought to promote the desquamation of the stratum corneum. (Fig. 1a). KLKs have also been reported to activate IL-1 in the skin [13]. In addition, an increase in pH enhances the serine protease activities [14].

In AD lesions, KLKs are overexpressed, and the pH of the stratum corneum is increased [15, 16]. Excessive expression and activities of KLKs disrupts the normal epidermal barrier function, and foreign substances such as AD antigens can easily invade the epidermis, thereby causing dermatitis (Fig.1b).

KLK5 and AD

KLK5 is the major tryptic serine protease in the epidermis and was first reported and cloned in 1999 as a stratum corneum tryptic enzyme (SCTE) [17]. KLK5 causes exfoliation of the stratum corneum by cleaving DSG1, DSC1, and CDSN (Fig. 1a) [12]. KLK5 is indirectly involved in the processing of (pro) filaggrin through the activation of elastase-2, a serine protease expressed in the epidermis [18]. It has been pointed out that KLK5 is localized not only in lamella granules but also in keratohyalin granules and may be directly involved in the processing of profilaggrin processing [19]. In addition, KLK5 activates the antimicrobial peptide LL-37 [20]. KLK5 is self-activated or activated by KLK14

[21].

KLK5 expression is increased in AD lesions, and trypsin-like serine protease activity is also enhanced in lesions, which is thought to suppress the epidermal barrier function [6, 16]. Expression of the transcription factor specificity protein 1 (Sp1) is significantly reduced in AD lesions, and Sp1 gene silencing increases KLK5 expression in epidermal keratinocytes; thus, decreased Sp1 expression may increase KLK5 in AD [22]. IL-4 has been reported to suppress the expression of Sp1 mRNA in cultured epidermal keratinocytes, but does not directly affect the expression of KLK5 in these cells [23, 24]. In addition, our previous study revealed that activated vitamin D3, retinoic acid, and high-concentrations of calcium enhance the expression of KLK5 in the cells [25]. Further analysis is needed to elucidate the detailed mechanisms underlying the increased expression of KLK5 in AD lesions.

In addition, KLK5 induces the expression of thymic stromal lymphopoietin (TSLP) in epidermal keratinocytes, which creates a Th2 environment via protease-activated receptor 2 (PAR2) (Fig.2) [26]. Furthermore, an increase in skin pH directly regulates KLK5 activity against PAR2 [27]. In a study by Buhl, model mice with epidermal overexpression of PAR2 showed spontaneously developing AD-like skin disease, which suggested the importance of serine proteases and PAR2 in the pathogenesis of AD [28]. PAR2 expression is enhanced on nerve fibers in AD lesions, and intracutaneous injection of endogenous PAR2 agonists provokes itching (Fig. 2) [29]. In addition, KLK5 has been reported to induce pruritus via PAR2 in a mouse model of AD (Fig. 2) [30]. Although

KLK5 can cleave PAR1 *in vitro*, there are no reports of studies on the association between KLK5 and PAR1 in AD [31]. However, Zhu et al. reported that KLK5 activation-induced AD-like skin structures are formed in a PAR2-independent manner [32]. In another study, a transgenic mouse model that overexpresses human KLK5 presented cutaneous and systemic hallmarks of severe inflammation and allergy with pruritus [33].

KLK7 and AD

KLK7 is the only chymotrypsin-like serine protease of the KLK family that is expressed in the epidermis, first reported in 1991 as a stratum corneum chymolytic enzyme (SCCE), and cloned in 1994 [34, 35]. KLK7 causes desquamation of the stratum corneum by cleaving DSC1 and CDSN (Fig. 1a) [12]. The increased pH in the stratum corneum observed in AD lesions enhances KLK7 activity [14, 15]. Also, KLK7 is also activated by KLK5 [21].

KLK7 expression is increased in AD lesions, and chymotrypsin-like serine protease activity is also enhanced in lesions, which is thought to reduce the epidermal barrier function, as observed in KLK5 (Fig. 1b) [6, 16]. On the other hand, there is a report that the expression of KLK7 is enhanced, but the enzymatic activity of epidermal keratinocytes taped from AD lesions is not increased [36]. Gene silencing of the transcription factor Sp1 also enhances KLK7 expression in epidermal keratinocytes [22]. Similar to KLK5, activated vitamin D₃, retinoic acid, and high-

concentrations of calcium also enhance KLK7 expression [25]. In addition, the Th2 cytokines IL-4 and IL-13 increase KLK7 expression and chymotrypsin-like serine protease activity in AD lesions [24]. Furthermore, serum KLK7 protein levels in patients with AD are significantly correlated with serum IL-4 levels [24]. KLK7 can degrade the antibacterial peptide LL-37 and reportedly reduce expression levels of LL-37 in AD lesions [20]. Transgenic mice overexpressing human KLK7 develop AD-like chronic pruritic dermatitis [37]. Reports suggest that KLK7 enhances itching independently of skin inflammation in AD, and unlike KLK5, KLK7 cannot activate PAR2 [38, 39].

Insertion of the AACC sequence in the 3'-UTR in KLK7 is significantly observed in patients with AD and has been reported to be involved in its increased mRNA expression [40, 41]. However, a French cohort study reported no significant association between this variant and AD [42].

Other KLKs and AD

KLK6, KLK8, KLK10, KLK11, KLK13, and KLK14 are tryptic-like serine proteases whose expression is increased in AD lesions [16]. KLK6, KLK8, and KLK14 are activated by KLK5 [21, 43]. The mechanism underlying KLK10 and KLK13 activation in epidermal keratinocytes has not been fully studied. KLK11 is activated by KLK8 [43].

Similar to KLK5, KLK6 and KLK14 also activate PAR2 [31]. Therefore, KLK6 and KLK14 may also be involved in the PAR2-mediated induction of inflammation and itching in AD (Fig. 2).

Gene silencing of the transcription factor Sp1 also increases the expression of KLK6, KLK8, and KLK10 in epidermal keratinocytes [22]. It has been reported that *Staphylococcus aureus* may be involved in the pathophysiology of AD by inducing the expression of KLK6, KLK13, and KLK14 and promoting the degradation of DSG1 and filaggrin [44].

In recent years, JAK-inhibiting internal and external drugs have become available for the treatment of AD, and the importance of JAK-STAT signaling in this disease has drawn attention. Yasuda et al. found that AD model mice (Spade mice) was induced by a gain-of-function mutation in the gene sequence of the "JAK1" molecule [45]. In addition, upon examining gene expression in the skin lesions of Spade mice, it was found that the expression of serine proteases Klk6 and marapsin was enhanced; additionally, the JAK1 mutation was also shown to enhance these protease expression *in vitro* experiments [45]. These findings suggest that enhanced JAK1 signaling may induce AD by inducing serine protease hyperactivity.

Furthermore, overexpression of KLK6 has been reported to induce psoriasis-like dermatitis and arthritis through the activation of PAR1 [46]. The involvement of KLK6-PAR1 axis in atopic dermatitis has not yet been reported, but future research on this topic is warranted.

LEKTI and AD

Epidermal barrier function is also altered by the dysfunction of serine protease inhibitors. LEKTI is a

major serine protease inhibitor produced by epidermal keratinocytes, and has a structure wherein 15 domains that inhibit enzyme activity are linked in a row [47]. The importance of LEKTI in the human epidermis is found in patients with Netherton syndrome (NS) [47]. NS is an autosomal recessive disorder characterized by congenital ichthyosis, hair abnormalities, and atopic manifestation, with an incidence of less than 1 in 100,000 to 200,000. In patients with NS, the *SPINK5* gene, which encodes LEKTI, have mutations which generate the premature termination codons, resulting in the loss of LEKTI expression [47]. As a result, serine protease activities in the corneal layer cannot be inhibited, excessive exfoliation of the stratum corneum occurs, and AD-like skin symptoms are exhibited (Fig.1c). In *Spink5*-deficient mice, which are NS model animals, the skin barrier function is reduced, transepidermal water loss (TEWL) is increased, and the activities of KLK5 and KLK7 are increased [48].

The relationship between AD and *SPINK5* gene mutations/polymorphisms has been studied by multiple research groups. Among these gene polymorphisms, E420K and D386N have been reported to be single nucleotide polymorphisms (SNPs) that alter the degradation pattern of LEKTI proteins and reduce their ability to inhibit serine protease activities [49, 50]. When we examined the prevalence of E420K and D386N in patients with AD visiting our hospital and related facilities, no significant difference from healthy subjects was found, contrary to the results of previous reports [7]. It is suggested that the prevalence of SNPs in *SPINK5* varies by race and region, similarly to the

prevalence of FLG mutations, in AD patients.

We also focused on the regulatory mechanism of LEKTI expression in epidermal keratinocytes and found that high concentrations of calcium significantly enhance LEKTI expression at the transcriptional and protein levels [51]. The induction of LEKTI is calcium concentration-dependent and time-dependent [51]. In addition, Toll-like receptor-mediated signals and the inflammatory cytokines TNF- α and IL-17A were also found to significantly enhance LEKTI expression [52]. Furthermore, in our previous study on the expression of LEKTI in normal skin, AD lesions, and psoriasis lesions, interestingly, the expression of LEKTI was markedly enhanced in psoriasis lesions, while in AD lesions it was at the same level as in normal skin [52].

Conclusion

In this article, we have outlined aberrant serine protease activities in AD. Many KLKs expression is upregulated in AD lesions and LEKTI function is decreased in some AD patients with SNPs E420K and D386N of *SPINK5*. These findings may be associated with the pathogenesis of AD. Further analysis is needed to elucidate the detailed mechanism underlying the control of serine protease activities, which may lead to the development of new therapeutic and prophylactic agents for AD.

Author contributions

Conceptualization: SM; Supervision: MO; Visualization: SM, KS, HN; Writing - Original

Draft Preparation: SM; Writing - Review and Editing: SM, KS, HN, MO

Acknowledgements

We thank Yuka Kashiara for supporting our experiments relating to epidermal serine protease activities in AD and NS.

Funding

This work was supported by Grant/research support from: Mitsubishi Tanabe Pharma Co., Sanofi K.K., and Maruho Co., Ltd.

Conflict of interest

S. Morizane has received research support from Mitsubishi Tanabe Pharma Co., Sanofi K.K., and Maruho Co., Ltd., and honoraria for lectures from Eli Lilly Japan K.K., AbbVie GK, Pfizer Japan Inc., Torii Pharmaceutical Co.,Ltd, Sanofi K.K., and Maruho Co., Ltd.. D. The remaining authors state no conflict of interest.

References

- [1] Kabashima K: New concept of the pathogenesis of atopic dermatitis: interplay among the barrier, allergy, and pruritus as a trinity. *J Dermatol Sci* 70, (2013) 3-11.
- [2] McAleer MA, Irvine AD: The multifunctional role of filaggrin in allergic skin disease. *J Allergy Clin Immunol* 131, (2013) 280-291.
- [3] Palmer CN, Irvine AD, Terron-Kwiatkowski A, Zhao Y, Liao H, Lee SP, et al.: Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nat Genet* 38, (2006) 441-446.
- [4] Song CW, Tabachnick J, McCarron DJ, Jr.: A trypsin-like protease in guinea pig skin. *Experientia* 25, (1969) 1063-1064.
- [5] Rajka G: Experimental pruritus in the unaffected skin of patients with different itching dermatoses. *Acta Derm Venereol* 50, (1970) 270-272.
- [6] Voegeli R, Rawlings AV, Breternitz M, Doppler S, Schreier T, Fluhr JW: Increased stratum corneum serine protease activity in acute eczematous atopic skin. *Br J Dermatol* 161, (2009) 70-77.
- [7] Nomura H, Suganuma M, Takeichi T, Kono M, Isokane Y, Sunagawa K, et al.: Multifaceted Analyses of Epidermal Serine Protease Activity in Patients with Atopic Dermatitis. *Int J Mol Sci* 21, (2020).

- [8] Nomura H, Kawakami Y, Yasutomi Y, Morizane S: Two atopic dermatitis patients in whom dupilumab improved aberrant epidermal protease activities. *J Cutan Immunol Allergy* 2, (2019) 87-88.
- [9] Kraut H, Frey E, Werle E: Der Nachweis eines Kreislaufhormon in der pankreasdruse. *Hoppe-Seyler Z Physiol Chem* 189, (1930) 97-106.
- [10] Yousef GM, Diamandis EP: The new human tissue kallikrein gene family: structure, function, and association to disease. *Endocr Rev* 22, (2001) 184-204.
- [11] Kalinska M, Meyer-Hoffert U, Kantyka T, Potempa J: Kallikreins - The melting pot of activity and function. *Biochimie* 122, (2016) 270-282.
- [12] Caubet C, Jonca N, Brattsand M, Guerrin M, Bernard D, Schmidt R, et al.: Degradation of corneodesmosome proteins by two serine proteases of the kallikrein family, SCCE/KLK5/hK5 and SCCE/KLK7/hK7. *J Invest Dermatol* 122, (2004) 1235-1244.
- [13] Nylander-Lundqvist E, Egelrud T: Formation of active IL-1 beta from pro-IL-1 beta catalyzed by stratum corneum chymotryptic enzyme in vitro. *Acta Derm Venereol* 77, (1997) 203-206.
- [14] Cork MJ, Robinson DA, Vasilopoulos Y, Ferguson A, Moustafa M, MacGowan A, et al.: New perspectives on epidermal barrier dysfunction in atopic dermatitis: gene-environment interactions. *J Allergy Clin Immunol* 118, (2006) 3-21; quiz 22-23.
- [15] Rippke F, Schreiner V, Doering T, Maibach HI: Stratum corneum pH in atopic dermatitis:

impact on skin barrier function and colonization with *Staphylococcus Aureus*. *Am J Clin Dermatol* 5, (2004) 217-223.

[16] Komatsu N, Saijoh K, Kuk C, Liu AC, Khan S, Shirasaki F, et al.: Human tissue kallikrein expression in the stratum corneum and serum of atopic dermatitis patients. *Exp Dermatol* 16, (2007) 513-519.

[17] Brattsand M, Egelrud T: Purification, molecular cloning, and expression of a human stratum corneum trypsin-like serine protease with possible function in desquamation. *J Biol Chem* 274, (1999) 30033-30040.

[18] Bonnart C, Deraison C, Lacroix M, Uchida Y, Besson C, Robin A, et al.: Elastase 2 is expressed in human and mouse epidermis and impairs skin barrier function in Netherton syndrome through filaggrin and lipid misprocessing. *J Clin Invest* 120, (2010) 871-882.

[19] Sakabe J, Yamamoto M, Hirakawa S, Motoyama A, Ohta I, Tatsuno K, et al.: Kallikrein-related peptidase 5 functions in proteolytic processing of profilaggrin in cultured human keratinocytes. *J Biol Chem* 288, (2013) 17179-17189.

[20] Yamasaki K, Schaubert J, Coda A, Lin H, Dorschner RA, Schechter NM, et al.: Kallikrein-mediated proteolysis regulates the antimicrobial effects of cathelicidins in skin. *FASEB J* 20, (2006) 2068-2080.

[21] Eissa A, Diamandis EP: Human tissue kallikreins as promiscuous modulators of homeostatic

skin barrier functions. *Biol Chem* 389, (2008) 669-680.

[22] Bin L, Kim BE, Hall CF, Leach SM, Leung DY: Inhibition of transcription factor specificity protein 1 alters the gene expression profile of keratinocytes leading to upregulation of kallikrein-related peptidases and thymic stromal lymphopoietin. *J Invest Dermatol* 131, (2011) 2213-2222.

[23] Kamata Y, Yamamoto M, Kawakami F, Tsuboi R, Takeda A, Ishihara K, et al.: Bleomycin hydrolase is regulated biphasically in a differentiation- and cytokine-dependent manner: relevance to atopic dermatitis. *J Biol Chem* 286, (2011) 8204-8212.

[24] Morizane S, Yamasaki K, Kajita A, Ikeda K, Zhan M, Aoyama Y, et al.: TH2 cytokines increase kallikrein 7 expression and function in patients with atopic dermatitis. *J Allergy Clin Immunol* 130, (2012) 259-261 e251.

[25] Morizane S, Yamasaki K, Kabigting FD, Gallo RL: Kallikrein expression and cathelicidin processing are independently controlled in keratinocytes by calcium, vitamin D(3), and retinoic acid. *J Invest Dermatol* 130, (2010) 1297-1306.

[26] Briot A, Deraison C, Lacroix M, Bonnart C, Robin A, Besson C, et al.: Kallikrein 5 induces atopic dermatitis-like lesions through PAR2-mediated thymic stromal lymphopoietin expression in Netherton syndrome. *J Exp Med* 206, (2009) 1135-1147.

[27] Jang H, Matsuda A, Jung K, Karasawa K, Matsuda K, Oida K, et al.: Skin pH is the Master Switch of Kallikrein 5-Mediated Skin Barrier Destruction in a Murine Atopic Dermatitis Model. *J*

Invest Dermatol, (2015).

[28] Buhl T, Ikoma A, Kempkes C, Cevikbas F, Sulk M, Buddenkotte J, et al.: Protease-Activated Receptor-2 Regulates Neuro-Epidermal Communication in Atopic Dermatitis. *Front Immunol* 11, (2020) 1740.

[29] Steinhoff M, Neisius U, Ikoma A, Fartasch M, Heyer G, Skov PS, et al.: Proteinase-activated receptor-2 mediates itch: a novel pathway for pruritus in human skin. *J Neurosci* 23, (2003) 6176-6180.

[30] Andoh T, Tsujii K, Kuraishi Y: Increase in pruritogenic kallikrein 5 in the skin of NC mice with chronic dermatitis. *Exp Dermatol* 24, (2015) 978-980.

[31] Oikonomopoulou K, Hansen KK, Saifeddine M, Tea I, Blaber M, Blaber SI, et al.: Proteinase-activated receptors, targets for kallikrein signaling. *J Biol Chem* 281, (2006) 32095-32112.

[32] Zhu Y, Underwood J, Macmillan D, Shariff L, O'Shaughnessy R, Harper JI, et al.: Persistent kallikrein 5 activation induces atopic dermatitis-like skin architecture independent of PAR2 activity. *J Allergy Clin Immunol* 140, (2017) 1310-1322 e1315.

[33] Furio L, de Veer S, Jaillet M, Briot A, Robin A, Deraison C, et al.: Transgenic kallikrein 5 mice reproduce major cutaneous and systemic hallmarks of Netherton syndrome. *J Exp Med* 211, (2014) 499-513.

[34] Lundstrom A, Egelrud T: Stratum corneum chymotryptic enzyme: a proteinase which may

be generally present in the stratum corneum and with a possible involvement in desquamation.

Acta Derm Venereol 71, (1991) 471-474.

[35] Hansson L, Stromqvist M, Backman A, Wallbrandt P, Carlstein A, Egelrud T: Cloning, expression, and characterization of stratum corneum chymotryptic enzyme. A skin-specific human serine proteinase. J Biol Chem 269, (1994) 19420-19426.

[36] Igawa S, Kishibe M, Minami-Hori M, Honma M, Tsujimura H, Ishikawa J, et al.: Incomplete KLK7 Secretion and Upregulated LEKTI Expression Underlie Hyperkeratotic Stratum Corneum in Atopic Dermatitis. J Invest Dermatol 137, (2017) 449-456.

[37] Hansson L, Backman A, Ny A, Edlund M, Ekholm E, Ekstrand Hammarstrom B, et al.: Epidermal overexpression of stratum corneum chymotryptic enzyme in mice: a model for chronic itchy dermatitis. J Invest Dermatol 118, (2002) 444-449.

[38] Guo CJ, Mack MR, Oetjen LK, Trier AM, Council ML, Pavel AB, et al.: Kallikrein 7 Promotes Atopic Dermatitis-Associated Itch Independently of Skin Inflammation. J Invest Dermatol 140, (2020) 1244-1252 e1244.

[39] Stefansson K, Brattsand M, Roosterman D, Kempkes C, Bocheva G, Steinhoff M, et al.: Activation of proteinase-activated receptor-2 by human kallikrein-related peptidases. J Invest Dermatol 128, (2008) 18-25.

[40] Vasilopoulos Y, Cork MJ, Murphy R, Williams HC, Robinson DA, Duff GW, et al.: Genetic

association between an AACC insertion in the 3'UTR of the stratum corneum chymotryptic enzyme gene and atopic dermatitis. *J Invest Dermatol* 123, (2004) 62-66.

[41] Vasilopoulos Y, Sharaf N, di Giovine F, Simon M, Cork MJ, Duff GW, et al.: The 3'-UTR AACCins5874 in the stratum corneum chymotryptic enzyme gene (SCCE/KLK7), associated with atopic dermatitis; causes an increased mRNA expression without altering its stability. *J Dermatol Sci* 61, (2011) 131-133.

[42] Hubiche T, Ged C, Benard A, Leaute-Labreze C, McElreavey K, de Verneuil H, et al.: Analysis of SPINK 5, KLK 7 and FLG genotypes in a French atopic dermatitis cohort. *Acta Derm Venereol* 87, (2007) 499-505.

[43] Eissa A, Amodeo V, Smith CR, Diamandis EP: Kallikrein-related peptidase-8 (KLK8) is an active serine protease in human epidermis and sweat and is involved in a skin barrier proteolytic cascade. *J Biol Chem* 286, (2011) 687-706.

[44] Williams MR, Nakatsuji T, Sanford JA, Vrbanc AF, Gallo RL: *Staphylococcus aureus* Induces Increased Serine Protease Activity in Keratinocytes. *J Invest Dermatol* 137, (2017) 377-384.

[45] Yasuda T, Fukada T, Nishida K, Nakayama M, Matsuda M, Miura I, et al.: Hyperactivation of JAK1 tyrosine kinase induces stepwise, progressive pruritic dermatitis. *J Clin Invest* 126, (2016) 2064-2076.

[46] Billi AC, Ludwig JE, Fritz Y, Rozic R, Swindell WR, Tsoi LC, et al.: KLK6 expression in skin

induces PAR1-mediated psoriasiform dermatitis and inflammatory joint disease. *J Clin Invest* 130, (2020) 3151-3157.

[47] Hovnanian A: Netherton syndrome: skin inflammation and allergy by loss of protease inhibition. *Cell Tissue Res* 351, (2013) 289-300.

[48] Descargues P, Deraison C, Bonnart C, Kreft M, Kishibe M, Ishida-Yamamoto A, et al.: Spink5-deficient mice mimic Netherton syndrome through degradation of desmoglein 1 by epidermal protease hyperactivity. *Nat Genet* 37, (2005) 56-65.

[49] Fortugno P, Furio L, Teson M, Berretti M, El Hachem M, Zambruno G, et al.: The 420K LEKTI variant alters LEKTI proteolytic activation and results in protease deregulation: implications for atopic dermatitis. *Hum Mol Genet* 21, (2012) 4187-4200.

[50] Ramesh K, Matta SA, Chew FT, Mok YK: Exonic mutations associated with atopic dermatitis disrupt lympho-epithelial Kazal-type related inhibitor action and enhance its degradation. *Allergy* 75, (2020) 403-411.

[51] Kobashi M, Morizane S, Sugimoto S, Sugihara S, Iwatsuki K: Expression of serine protease inhibitors in epidermal keratinocytes is increased by calcium but not 1,25-dihydroxyvitamin D3 or retinoic acid. *Br J Dermatol* 176, (2017) 1525-1532.

[52] Sugihara S, Sugimoto S, Tachibana K, Kobashi M, Nomura H, Miyake T, et al.: TNF-alpha and IL-17A induce the expression of lympho-epithelial Kazal-type inhibitor in epidermal

keratinocytes. J Dermatol Sci 96, (2019) 26-32.

Figure legends

Figure 1. (a) KLKs and LEKTI in normal skin. The epidermal serine protease activities are mainly controlled by KLKs and LEKTI. KLK5 and KLK7 promote desquamation of the corneum. Below the stratum granulosum, LEKTI tightly regulates the activity of KLKs. **(b) KLKs and LEKTI in AD lesions.** In AD lesions, KLK expression is increased, which results in the enhancement of epidermal serine protease activities and facilitates the invasion by allergens and microorganisms. **(c) KLKs and LEKTI in NS.** In NS lesions, LEKTI expression is deficient, resulting in the activation of KLKs and easy invasion by allergens and microorganisms. Kallikrein-related peptidases (KLKs); lymphoepithelial Kazal-type-related inhibitor (LEKTI); Atopic dermatitis (AD) ; Netherton syndrome (NS)

(NS)  KLK5  KLK7  LEKTI

Figure 2. KLKs activates PAR2

KLK5, 6, and 14 can activate PAR2 in epidermal keratinocytes and peripheral nerves, which results in the induction of inflammation and itching. kallikrein-related peptidases (KLKs); protease-activated receptor 2 (PAR2)