Kallikrein 7 Promotes Atopic Dermatitis-Associated Itch Independently of Skin Inflammation

Changxiong J. Guo1,2,5, Madison R. Mack2,3,5, Landon K. Oetjen2,3, Anna M. Trier2,3, Martha L. Council3, Ana B. Pavel4, Emma Guttman-Yassky4, Brian S. Kim1,2,3 and Qin Liu1,2

Atopic dermatitis (AD) is a highly prevalent, itchy inflammatory skin disorder that is thought to arise from a combination of skin barrier defect and immune dysregulation. Kallikreins (KLK), a family of serine proteases with a diverse array of homoeostatic functions, including skin desquamation and innate immunity, are hypothesized to contribute to AD pathogenesis. However, their precise role in AD has not been clearly defined. In this study, RNA sequencing analyses identified KLK7 as the most abundant and differentially expressed KLK in both human AD and murine AD-like skin. Further, in mice, KLK7 expression was localized to the epidermis in both steady state and inflammation. Unexpectedly, KLK7 was dispensable for the development of AD-associated skin inflammation. Instead, KLK7 was selectively required for AD-associated chronic itch. Even without the alleviation of skin inflammation, KLK7-deficient mice exhibited significantly attenuated scratching, compared with littermate controls, after AD-like disease induction. Collectively, our findings indicate that KLK7 promotes AD-associated itch independently from skin inflammation and reveal a previously unrecognized epidermal-neural mechanism of AD associated itch.


INTRODUCTION

Atopic dermatitis (AD) is a chronic and relapsing inflammatory skin disorder that affects 10–20% of the population (DaVeiga, 2012; Silverberg, 2017). In moderate or severe forms, the crusted, oozing, and itchy skin lesions can dramatically lower the quality of life for patients with AD (Drucker et al., 2017). Although chronic itch (pruritus) is considered the central and most debilitating symptom of AD, treatments have almost exclusively targeted pro-inflammatory mediators, rather than specific pruritogenic pathways.

AD is currently thought to arise from a combination of skin barrier dysfunction and immune dysregulation (Brunner et al., 2018; Czarnowicki et al., 2019; Elias et al., 2008). Many recent studies have focused on how epidermal barrier defects (e.g., filaggrin mutations) lead to the initiation of allergic inflammation, characterized by the recruitment and activation of T helper type 2 cells, group 2 innate lymphoid cells, and basophils in the affected skin (Esaki et al., 2015; Kim, 2015; Mashiko et al., 2017; Palmer et al., 2006). These cells, in turn, produce the hallmark type 2 effector cytokines, IL-4, IL-5, and IL-13, which are central to AD immune dysregulation (Brandt and Sivaprasad, 2011; Guttman-Yassky et al., 2019). However, despite our increasing understanding of the inflammatory pathogenesis of AD, the molecular mechanisms of AD-associated itch remain poorly defined.

The kallikrein (KLK) family of serine proteases is hypothesized to be a significant contributor to AD pathology. A number of KLKs are reported to be enriched in the lesional AD skin of humans (Komatsu et al., 2007, 2005; Morizane et al., 2012; Vasilopoulos et al., 2011) and may contribute to AD-associated pruritus in patients (Nattkemper et al., 2018). Moreover, the overexpression of KLKs in murine skin can spontaneously lead to an AD-like disease (Briot et al., 2009; Hansson et al., 2002).

In humans, the KLK family is comprised of 15 structurally conserved members (KLK1–15) that are involved in an array of homoeostatic and disease processes across a wide variety of tissues (Shaw and Diamandis, 2007; Sotropoulou et al., 2009). In healthy human skin, serine peptidase activity in the stratum corneum is mainly attributed to the trypsin-like KLK5 (Deraison et al., 2007; Ekholm et al., 2000) and the chymotrypsin-like KLK7 (Borgoño et al., 2007; Caubet et al., 2004; Yousef et al., 2000), which help maintain barrier homoeostasis by regulating the cleavage of corneodesmosomes. Notwithstanding this, a number of other KLKs may also be present in lower abundance and have related functions (Borgoño et al., 2007; Komatsu et al., 2007, 2005, 2003).
All KLKs are translated as pre-pro-enzymes and secreted into the extracellular space as inactivezymogens. There, they are activated by a variety of mechanisms, including autocalytic activity, endopeptidases, or by other KLKs (Sotropoupolou et al., 2009). In the skin, KLK activity is further controlled by a number of homeostatic processes, including the endogenous activity of serine protease inhibitors (serpins), such as lymphoepithelial Kazal-type inhibitor encoded by SPINK5 (Deraison et al., 2007). In the setting of AD, it is hypothesized that the over activity of epidermal KLKs set off a cascade of proteolytic activity, which in turn contributes to barrier defects and AD pathogenesis.

In lesional AD skin, a number of KLKs, including KLK5 and KLK7, have been shown to be increased (Brunner et al., 2017; Komatsu et al., 2007; Morizane et al., 2012; Vasilopoulos et al., 2011). Furthermore, the transgenic overexpression of Klk5 or Klk7 in mice resulted in the spontaneous development of AD-like disease features (Briot et al., 2009; Furio et al., 2014; Hansson et al., 2002). Likewise, loss-of-function mutations in SPINK5 resulted in unregulated epidermal KLK activity and Netherton Syndrome, a severe AD-like syndrome, in both mice and humans (Chavanas et al., 2000; Descargues et al., 2005). In mice, the symptoms of Netherton Syndrome can be prevented by the genetic ablation of Klk5 and Klk7 (Kasperek et al., 2017). Although it is becoming increasingly clear that KLK dysregulation contributes to AD pathogenesis, the mechanisms underlying this process remain poorly defined.

In this study, we demonstrate that KLK7, but not KLK5, is upregulated in human and murine lesional AD skin. Further, we show that basal and AD-associated KLK7 expression is restricted to the epidermis, provoking the hypothesis that epithelial cell-derived KLK7 is critically required for the development AD. Surprisingly, we found that KLK7-deficient mice had no improvement in AD-like skin disease severity but showed markedly attenuated AD-associated itch. These findings demonstrate a previously unrecognized role for KLK7 in mediating itch in the context of AD, and provide additional insight into KLK function in disease states.

RESULTS

KLK7 is upregulated in human AD lesions

Both human AD and murine AD-like disease are characterized by several shared histological and immunological features. At the molecular level, these include a variety of KLKs upregulated in lesional AD skin (Komatsu et al., 2007). However, which KLKs are selectively and critically upregulated remains to be defined.

In this study, we reanalyzed a previously published RNA sequencing (RNA-Seq) dataset of human control and lesional AD skin for the top differentially expressed genes by fold change and expression level (Oetjen et al., 2017). Unbiased analysis of the most abundant differentially expressed genes identified KLK7 as the only KLK that was differentially upregulated in human AD skin (Figure 1a). Other keratinocyte-associated genes, such as the S100A family members, CAPN1, and CASP14, were also enriched in AD skin (Figure 1a). Comprehensive analysis of all the KLKs in this dataset demonstrated that, consistent with prior studies, KLK5 and KLK7 are the most highly expressed KLKs in control human skin, accounting for two-thirds of the total KLK transcripts in control biopsies (Figure 1b). In addition, four KLKs—KLK7, KLK8, KLK10, and KLK11—showed statistically significant expression increases in lesional AD skin, with KLK7 exhibiting the most prominent overexpression. Changes in KLK8, KLK10, and KLK11 expression were comparatively less pronounced. Surprisingly, KLK5 expression was unchanged.

Our finding that KLK7 is selectively overexpressed in lesional AD skin compared with KLK5 was further confirmed using additional sets of human control and AD skin samples. Using quantitative reverse transcriptase-PCR (RT-qPCR), we confirmed that KLK7, but not KLK5, was significantly

**Figure 1. KLK7 is overexpressed in human lesional AD skin.** (a) Heatmap of z-scored RPKMs of differentially expressed genes with > 2-fold change, adjusted P < 0.05, and base mean expression level > 1,000. (b) RPKMs of all human KLKs present in skin samples of control and AD individuals. N = 4 per group. (c–e) RT-qPCR of (c) KLK5 and (d) KLK7 in human control and AD skin, normalized to (e) ACTB. N = 8–10 per group. Error bars = standard error of the mean. *P < 0.05. **P < 0.01. AD, atopic dermatitis; KLK, kallikrein; n.s, no significance; RPKM, reads per kilobase of transcript, per million mapped reads; RT-qPCR, quantitative reverse transcriptase–PCR.
enriched in lesional AD skin (Figure 1c−e). Additionally, we looked specifically at matched lesional and nonlesional sites in another cohort of 35 patients with AD by RNA-Seq (Supplementary Figure S1a and b). Consistent with the observations from our other cohorts, we found no increases in Klk5 expression in lesional AD skin in this dataset (Supplementary Figure S1a). Moreover, we confirmed that Klk7 mRNA is significantly elevated in lesional AD skin, compared with matched nonlesional sites (Supplementary Figure S1b). However, the variations in Klk7 levels within lesional tissues did not significantly correlate with patient-reported itch severity (Supplementary Figure S1c). Based on these results, we hypothesized that Klk7 may be a key driver in AD disease pathogenesis.

**Klk7 is upregulated in murine AD-like lesions**

Since Klks are highly conserved across mammalian species (Pavlopoulou et al., 2010), we explored whether Klk7 expression is also dysregulated in murine AD-like skin. To test this, we employed a well-established model of AD-like disease, in which mice are treated with the topical irritant MC903 (calcipotriol) (Li et al., 2006) (Figure 2a). Following MC903 treatment, mice develop the histopathologic features of AD (Figure 2b), as well as chronic itch behavior (Figure 2c).

To define the expression status of Klk7 in mouse AD-like skin, we reanalyzed a previously published RNA-Seq dataset of MC903-treated murine AD-like skin (Oetjen et al., 2017). Using the same unbiased analysis technique, we identified Klk7 among the top most abundant differentially expressed genes in murine AD-like skin (Figure 2d). A comprehensive analysis of all the Klks in this dataset demonstrated that, like human skin (Figure 1), Klk7 was the most highly expressed Klk in control skin, accounting for 66.9% \( \pm 1.43% \) of the total Klk transcripts. Moreover, seven Klks − Klk6, Klk7, Klk8, Klk9, Klk10, Klk11, and Klk13 − showed statistically significant increases in expression in murine AD-like skin (Figure 2e). Similar to our findings in human AD skin, Klk7 was the most abundant transcript and demonstrated a four-fold increase in expression in AD-like skin (Figure 2e). Klk5 expression, again, was unchanged in this context (Figure 2e). These findings were further validated with additional samples by RT-qPCR. Klk7, but not Klk5, was significantly enriched in AD-like skin (Figure 2f−h).
Based on the consistent KLK7 upregulation in AD skin of both humans and mice, we sought to investigate the mechanisms by which KLK7 promotes AD-like disease pathogenesis in vivo.

**Epidermal KLK7 expression is enhanced in AD-like disease and promotes itch but not inflammation**

KLK7 expression is highly conserved between mice and humans and is mainly restricted to the skin in both species (Fagerberg et al., 2014; Pavlopoulou et al., 2010). In the steady state, murine Klk7 expression is largely restricted to epidermal skin and is undetectable in dermal skin, neural, and immune tissues (Figure 3a and Supplementary Figure S2). After the induction of AD-like disease, Klk7, detected using a LacZ-β-galactosidase reporter, was selectively enhanced in the epidermis, but remained undetectable in the dermis where infiltrating immune cells and peripheral fibers of sensory neurons are present (Figure 3a). Taken together, these findings indicate that the epidermis is the primary source of Klk7 in the skin, in both homeostatic and inflammatory conditions.

**Figure 3. Klk7 deficiency does not cause defects in AD-associated immune response.** (a) X-gal staining of nape skin from Klk7LacZ mice without treatment (control) and after treatment with MC903. (b) Representative images of AD-like disease induction in control (wild-type) and Klk7−/− mice after 6 days of MC903 treatment; N ≥ 8 mice per group. (c) Representative H&E-stained sections of MC903-treated skin of control and Klk7−/− mice; N = 4 mice per group. (d) Histological grading of H&E-stained sections of control and Klk7−/− mouse skin; N = 4 mice per group. (e-i) Flow cytometric analysis of AD-associated (e) ILC2, (f) eosinophil, (g) mast cell, (h) basophil, and (i) Th2 cell frequency in MC903-treated nape skin from control and Klk7−/− mice on day 6 of treatment; n = 5 mice per group. Error bars = standard error of the mean. Scale bars = 200 μm. AD, atopic dermatitis; H&E, hematoxylin and eosin; ILC2, group 2 innate lymphoid cell; n.s., no significance; Th2, T helper type 2; WT, wild-type; X-gal, 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside.
Additional quantification of T helper type 2 cells and inflammatory markers did not reveal any detectable differences between MC903-treated control and Klk7−/− mice. Serum IgE and TARC/CCL17 levels, as detected by ELISA, were similar between the genotypes (Supplementary Figure S3a–b). The Il4ra, Il4, and Il13 expression levels in MC903-treated lesional skin, as detected by RT-qPCR, were also not different between the control and Klk7−/− mice (Supplementary Figure S3c–e). Lastly, Tsp1 expression in the skin was strongly induced by MC903 treatment as expected, but was expressed at similar levels between the genotypes at both basal and diseased states (Supplementary Figure S3f).

Despite the lack of observable differences in skin inflammation and key inflammatory markers, spontaneous scratching was markedly reduced in Klk7−/− mice, when compared with controls (Figure 4a). On average, Klk7−/− mice scratched two to three times less than the controls and demonstrated a mean of 61.33 ± 10.24 bouts per 30 minutes, versus 186.13 ± 25.89 bouts per 30 minutes for the control mice on day 6 of treatment (Figure 4a). These findings were recapitulated in a 12-day ear treatment model, in which Klk7−/− mice exhibited a similar reduction in itch (Supplementary Figure S4).

Given the striking itch phenotype, we examined whether sensory neuron activation was correspondingly attenuated in Klk7−/− mice under AD-like conditions. Although rarely detectible in primary afferents at baseline, mitogen-activated protein kinase 3/1 phosphorylation can be transiently induced in dorsal root ganglia (DRG) neurons by intense stimulation or under chronic pathological conditions (Gao and Ji, 2009). Upper thoracic DRGs that innervate the inflamed skin showed a substantially diminished proportion of phosphorylated mitogen-activated protein kinase 3/1 immunoreactive neurons in Klk7−/− mice, compared with the control mice (2.11 ± 0.34% vs 4.00 ± 0.60%, respectively) (Figure 4b–c), indicating that the activation and sensitization of peripheral sensory neurons were significantly attenuated in Klk7−/− mice. Likewise, c-Fos expression, which is induced by the strong and sustained activation of spinal dorsal horn neurons (Gao and Ji, 2009), was significantly decreased in the thoracic spinal cords of Klk7−/− mice, most notably in lamina I and II where itch-sensing c-fiber DRG neurons project (Han et al., 2013) (Figure 4d–e). These findings indicate that, along with a reduction in behavioral itch (Figure 4a), sensory neuron activation is significantly attenuated in Klk7−/− mice in the context of AD. Collectively, our findings demonstrate that the epidermis is the major source of Klk7 and reveal a previously unrecognized epithelial-neural circuit by which Klk7 specifically mediates AD-associated itch.

**DISCUSSION**

KLKs are highly conserved across species, widely expressed throughout many organ systems, and exhibit heterogeneity in their composition across tissues (Pavlopoulou et al., 2010; Sotiropoulou et al., 2009). As serine proteases, they are involved in a diversity of both homeostatic and pathologic processes. In the skin, their primary role is to
mediate desquamation (Ekholm et al., 2000). Both KLK5 and KLK7 have been shown to be key mediators of corneodesmosomal cleavage and epithelial turnover (Caubet et al., 2004). While a number of KLKs, including KLK5 and KLK7, have been shown to be upregulated in lesional AD skin from patients (Brunner et al., 2018; Komatsu et al., 2004). While a number of KLKs, including KLK5 and KLK7 have been shown to be upregulated in lesional AD skin of both mice and humans. Second, we show that, despite its abundant expression in the skin, KLK7 is dispensable for the development of AD-associated inflammation. Third, the epidermal expression of KLK7 is selectively required for AD-associated itch. Importantly, these findings demonstrate an emerging paradigm in itch biology, that itch mediators may be separate from the mechanism driving AD-like skin inflammation.

The induction of the type 2 cytokines IL-4 and IL-13 critically promote AD-associated skin inflammation and itch (Oetjen and Kim, 2018; Trier and Kim, 2018). Additionally, clinical trials with dupilumab, an anti-IL-4Rα monoclonal antibody, have demonstrated rapid and marked improvement of itch symptoms in patients with AD (Beck et al., 2014; Guttmann-Yassky et al., 2019; Simpson et al., 2016; Thaci et al., 2016). Recent studies have shown that both IL-4 and IL-13 induce selective expression of KLK7 but not KLK5 in normal human epidermal keratinocyte cells (Morizane et al., 2012). Thus, whether there is a type 2 cytokine-epithelial-KLK circuit promoting itch remains a promising area of future inquiry.

Although prior studies have reported the broad upregulation of a number of KLKs in AD skin (Komatsu et al., 2007; Vasilopoulos et al., 2011), our gene expression analysis only identified KLK7 as the predominant KLK in both mouse and human AD skin. Despite previous reports that KLK5 is upregulated in human AD skin (Komatsu et al., 2007) and implicated in the promotion of AD-like disease in mice (Briot et al., 2009; Furio et al., 2014), we consistently could not detect KLK5 upregulation in AD skin. This may be in part due to the complexity of AD disease, with varying stages (acute vs chronic), levels of severity, and genetic- and age-dependent heterogeneity. For example, mutations in filagrin vary considerably across ethnicities, and there is emerging evidence that immune profiles in AD are also sensitive to the genetic background of individuals (Czarnowicki et al., 2019; Kaufman et al., 2018; Leung, 2015; Margolis et al., 2014; Osawa et al., 2011). Future studies focused on understanding the differential contributions of KLK5 and KLK7 to skin inflammation and itch are therefore warranted. Additionally, it is widely appreciated that KLKs can activate one another (Sotiropoulou et al., 2009). Thus, understanding how KLK7 may interact with other KLKs to regulate the development of AD-associated itch is an intriguing area of investigation.

Prior studies have shown that the overexpression of Klk7 was particularly notable for the development of severe itch associated with cutaneous inflammation (Hansson et al., 2002). Despite these advances, whether KLK-mediated itch occurs indirectly through the induction of skin inflammation or directly by acting on sensory neurons was unknown. Our finding that Klk7 deficiency attenuates AD-associated itch with no effect on inflammation provokes the hypothesis that this phenomenon occurs through direct epidermal-neuronal mechanisms. It has previously been shown that KLK5 can proteolytically activate PAR2 receptors, which have been heavily implicated in itch (Liu et al., 2011; Shimada et al., 2006; Stefansson et al., 2008). However, Klk7 does not exhibit this function (Supplementary Figure S5) (Stefansson et al., 2008) or the ability to induce significant behavioral response or neuronal activation in naïve mice (Supplementary Figure S6a–c). Thus, whether KLK7 generates endogenous pruritogens or if Klk7 can proteolytically sensitize neuronal itch receptors in AD skin remains open to further exploration.

In conclusion, therapeutic agents targeting KLK7 may be able to provide substantial itch relief for patients with AD. Owing to the specificity of KLK7 expression in epidermal skin, KLK7 antagonists could be used topically on affected AD skin to avoid potential side effects. Moreover, given the unique chymotrypsin-like functionality of KLK7, in contrast to the trypsin-like properties of other KLKs, redundancy and compensation by other KLKs is also less likely following pharmacologic KLK7 inhibition. These findings open exciting avenues for the exploration and development of novel therapeutics that target KLKs in the setting of AD and its associated itch.

**MATERIALS AND METHODS**

**Animals**

C57BL/6Nj wild-type mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Klk7tm1(KOMP)Vlcg sperm was purchased from the UC Davis KOMP repository (Davis, CA). IVF was performed by the Washington University School of Medicine Molecular Genetics Service Core. PirtC14::CamP5/- mice were a gift from Dr. Xinzhong Dong at Johns Hopkins University. All researchers were blinded to mouse genotypes throughout testing and data quantification.

**Human sample collection**

AD diagnosis was made by a board-certified dermatologist using the American Academy of Dermatology recommended criteria (Eichenfield et al., 2014). Biopsy samples were collected from consenting patients in clinics. Control tissues were either obtained from individuals without a history of inflammatory skin conditions or from healthy skin margins from patients undergoing Mohs surgery. All the skin samples were de-identified and stored in RNAlater (ThermoFisher, Waltham, MA) at −80°C before processing.

**Mouse Tissue Collection**

For reverse transcriptase–PCR and RT-qPCR, the mice were euthanized via carbon dioxide overdose, and tissues were dissected and stored in ice cold RNaseasy Kit Lysis Buffer (Qiagen, Hilden, Germany). Samples were processed for RNA extraction immediately after dissection.

For histopathology and LacZ staining, the mice were transcardially perfused with ice cold phosphate buffered saline and 4% paraformaldehyde (PFA) for fixation. Dissected tissues were fixed on ice as follows: skin in 1% PFA for 1 hour, DRG and trigeminal ganglia in 2% PFA for 30 minutes, spinal cord in 2% PFA for 1 hour,
and brain in 2% PFA for 2 hours. After fixation, the tissues were immersed in 30% sucrose for 24 hours at 4°C and embedded in O.C.T. (Sakura, Torrance, CA) for frozen sectioning. Mouse DRGs and spinal cords used for immunostaining were fixed in 4% PFA on ice for 90 minutes before sucrose incubation and frozen sectioning. For flow cytometry, the skin was harvested without fixation and processed immediately.

For ELISA, mouse serum was isolated from blood collected by orbital bleed by centrifugation at 10,000g for 10 minutes at 4°C on experimental day 6. ELISAs for IgG (Biolegend 432404, San Diego, CA) and TARC/CCL17 (R&D DY529-05, Minneapolis, MN) were performed according to the manufacturers’ instructions.

**Hematoxylin and eosin, X-Gal, and immunofluorescence staining**

O.C.T.-embedded mouse samples were sectioned on a Leica (Buffalo Grove, IL) cryostat. Brain sections were processed as floating sections, whereas other tissues were slide-mounted. Hematoxylin and eosin staining was performed by the Washington University School of Medicine Pulmonary Morphology Core. Images were acquired using a BX63 microscope (Olympus, Waltham, MA). The clinical grade was determined using a previously published protocol (Kim et al., 2014).

The tissues used for LacZ and immunofluorescence staining were allowed to air dry for 2 hours before processing. For LacZ staining, the slides were fixed on ice in 1% PFA for 1 minute. Chromogenesis was performed using the X-Gal Staining Assay Kit (Genlanolis, San Diego, CA) according to the manufacturer’s instructions. The color development time was approximately 6 minutes. Sections were dehydrated and mounted using ThermoFisher Permount.

For phosphorylated mitogen-activated protein kinase 3/1 and c-FOS immunofluorescence staining, the slides were blocked using 10% normal goat serum (MilliporeSigma, Burlington, MA) and incubated in primary antibodies overnight at 4°C. Afterwards, the slides were incubated in fluorescent secondary antibody for 2 hours at room temperature and mounted using Fluoromount-G (SouthernBiotech, Birmingham, AL). Images were acquired using a Ti-E microscope (Nikon, Melville, NY). The antibodies used were rabbit anti–phospho-p44/42 mitogen-activated protein kinase (Th2r202/Tyr204) monoclonal antibody (CST, Danvers, MA), rabbit anti–c-Fos polyclonal antibody (Calbiochem, San Diego, CA), goat anti-Rabbit IgG (H+L), and Alexa Fluor 488 conjugated secondary (ThermoFisher, Waltham, MA). All the antibodies were diluted 1:1,000 in phosphate buffered saline with 1% Tween-20 and 1% normal goat serum.

**RNA isolation, reverse transcriptase—PCR, and RT-qPCR**

RNA-Seq was performed and analyzed as previously described (Oetjen et al., 2017). Briefly, for RNA-Seq, 1 μg of total RNA was enriched with RiboZERO (Illumina, San Diego, CA) and sequenced on an Illumina HiSeq3000. Sequences were aligned with STAR (Dobin et al., 2013), counted with Subread:featureCount (Liao et al., 2014), and differential gene expression was determined by the DESeq2 package (Love et al., 2014) in R version 3.5.

The samples for reverse transcriptase—PCR and RT-qPCR were homogenized in lysis buffer supplied with the Qiagen RNeasy Kit using a bead homogenizer. RNA extraction was performed using the same kit, according to the manufacturer’s instructions. Samples were then treated with ThermoFisher Turbo DNase, and cDNA was generated using approximately 1,000 ng of total RNA and an ABI High-Capacity cDNA Reverse Transcription Kit (ABI, Waltham, MA). Reactions without reverse transcriptase were included as negative controls for downstream PCR.

Reverse transcriptase—PCR was performed using Qiagen HotStarTaq Polymerase and 10 ng of template. The images presented are representative of the results of 30 PCR cycles. RT-qPCR was performed using ABI Fast Sybr Green Master Mix and a Step-One Plus Real-Time PCR System. All reactions were run as technical triplicates using 10 ng of cDNA template, and the presented data were normalized to ACTB (for human samples) or Gapdh (for mouse samples) expression. The primers used are listed in Supplementary Table S1.

**MC903 treatment and mouse behavior**

The murine AD model was adapted from previous publications (Kim et al., 2014, 2013; Li et al., 2006; Oetjen et al., 2017). In the nape model, the nape and lower back areas (approximately 17 mm × 17 mm each) were shaved under isoflurane anesthesia 3 days before baseline behavioral recording. Test animals were habituated for two consecutive days immediately preceding baseline recording. To induce AD-like disease, 40 μl of 0.1 mM MC903 in ethanol was applied topically to the shaved nape skin under anesthesia once every 24 hours, starting 24 hours after baseline recording, for six consecutive days. Spontaneous scratching was scored for four consecutive days, starting 4 days after the baseline recording. Scratching bouts, defined as a continuous scratch movement directed at the treated skin by the hind paw, were scored from video recordings after the completion of the experiment. For the ear model, mice were treated with 40 μl of 0.05 mM MC903 on each ear under anesthesia for 8 days. The ear thickness was determined daily with a dial caliper.

**Flow cytometry**

Flow cytometry was performed as previously described (Oetjen et al., 2017). Briefly, harvested tissues were digested in 0.25 mg/ml Liberase TL (Roche, Switzerland) in DMEM media for 90 minutes at 37°C. Afterwards, the tissues were mechanically dissociated and passed through 70 μm cell strainers to obtain a single cell suspension. The cells were then stained with ZombieUV (Biolegend) at room temperature for 20 minutes, followed by primary antibodies (Supplementary Table S2) on ice for 30 minutes. Biolegend streptavidin-FITC and streptavidin-PE secondary stains were performed on ice for 30 minutes. Samples were acquired on an LSRFortessa X-20 (BD, Franklin Lakes, NJ).

**Calcium imaging**

Calcium imaging was performed as previously described (Huang et al., 2018; Oetjen et al., 2017). In brief, KNKR cells stably expressing hPAR2 receptors (KNKR-PAR2) were seeded onto cover slips precoated with 0.1 mg/ml poly-D-lysine (Corning, Corning, NY) and cultured overnight in DMEM complete medium. Primary cultures of DRG neurons from Pir5±/− mice were acutely extracted and dissociated in dispase II and collagenase I enzyme mixture from ThermoFisher. Dissociated DRG neurons were then seeded onto coverslips precoated with poly-D-lysine and 0.01 mg/ml laminin (Corning) and cultured overnight in DH10 media supplemented with 25 pg/ml NGF (Corning) and 50 pg/ml GDNF (R&D Systems).

Approximately 24 hours after seeding, KNKR cells were loaded with Fura2-AM (Fisher) and imaged at 340 and 380 nm excitation. DRG neurons were imaged at 488 nm excitation. Images were
acquired using a Nikon (Melville, NY) Ti-E microscope with a Photometrics (Tucson, AZ) HQ2 camera. rhKLLK7 (R&D Systems) and trypsin (ThermoFisher) were bath applied. Ratios of 340/380 nm and quantified green fluorescent protein fluorescence were determined using Nikon NIS AR software.

Data analysis
All data are presented as the mean ± standard error of the mean. Statistical significance for the two groups was determined using a two-tailed, unpaired Student’s t test. The differences between three or more groups were tested using a one-way analysis of variance, followed by two-tailed Student’s t tests. Differences were considered significant if \( P < 0.05 \). Flow cytometry data was analyzed with TreeStar (Ashland, OR) Flowjo v10. Graphs were generated using Graphpad (San Diego, CA) Prism 8 and R version 3.5 (Vienna, Austria).

ETHICS STATEMENT
All the animal experiments were performed under protocols approved by the Institutional Animal Care and Use Committee at Washington University School of Medicine and in strict accordance with recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. Human studies were performed under protocols reviewed and approved by local Institutional Review Boards, and biopsies were taken only after obtaining written, informed patient consent.

Data availability statement
Datasets related to this article can be found at the GEO DataSets using accession numbers GSE90883 and GSE140380, hosted at www.ncbi.nlm.nih.gov.

ORCIDs
Changxiang J Guo: https://orcid.org/0000-0001-3551-9100
Madison R Mack: https://orcid.org/0000-0001-9426-9171
Landon K Oetjen: https://orcid.org/0000-0002-1734-3510
Martha L Council: https://orcid.org/0000-0002-2657-9060
Ana B Pavel: https://orcid.org/0000-0003-8234-9853
Emma Guttmann-Yassky: https://orcid.org/0000-0001-9363-324X
Brian S Kim: https://orcid.org/0000-0002-8108-7161
Qiu Lin: https://orcid.org/0000-0003-4333-4951

CONFLICT OF INTEREST
BSK has consulted for AbbVie, Inc., Concert Pharmaceuticals, Incyte Corporation, Merck TheraDev, and Pfizer, Inc. and has been an advisory board participant for Celgene Corporation, Kiniksa Pharmaceuticals, Menlo TheraDev, Regeneron Pharmaceuticals, Inc., Sanofi, and Theravance BioPharma. BSK is a stockholder of Gilead Sciences, Inc., and Mallinckrodt Pharmaceuticals and founder and Chief Scientific Officer of Nuogen Pharma, Inc. EG has received grants (paid to Mount Sinai Health System), consulting fees, and/or honoraria from AbbVie, Allergan, Anacor, Asana Bioscience, Bristol-Myers Squibb, Celgene Corporation, Celsus Therapeutics, Curadinn Pharma, Dermira, Eversense, Galderma, Genentech, Glenmark, Janssen Biotech, Kymab Limited, Kyowa Kirin, Lead Pharma, LEO Pharma, Merck Pharmaceuticals, Novartis, Pfizer, Regeneron, Sanofi, Sienna BioPharmaceuticals, Stiefel/GlaxoSmithKline, TheraDev, and Vitae. MLC has consulted for MDOutlook. MRM, LKO, AMT, CJG, ABP, and QL state no conflict of interest.

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AUTHOR CONTRIBUTIONS
Conceptualization: CJG, MRM, QL, BSK; Data curation: MRM; Formal Analysis: CJG, MRM, ABP; Funding Acquisition: QL, BSK, EG; Investigation: CJG, MRM, ABP, LKO, AMT; Methodology: CJG, MRM, QL, BSK; Resources: MRM, BSK, LKO, EG; Supervision: QL and BSK; Validation: MRM, BSK, ABP, EG; Visualization: CJG and MRM; Writing — Original Draft: CJG, MRM, QL, BSK; Writing — Review & Editing: CJG, MRM, AMT, QL, BSK, ABP, and EG.

SUPPLEMENTARY MATERIAL
Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2019.10.022.

REFERENCES

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Supplementary Figure S1. KLK7, but not KLK5, is upregulated in human lesional AD skin. (a) Log2 read CPM of KLK5 in control skin from donors without AD (N = 20, age = 36.8 ± 2.4 years, 42.9% female), and matched pairs of NL skin and LS from donors with AD (N = 35, age = 34.3 ± 2.5 years, 50% female). (b) Log2 CPM of KLK7 in the same RNA-Seq data set. Note that KLK7 is overexpressed only in LS from donors with AD. *P < 0.05. **P < 0.01. (c) Pearson correlation between KLK7 transcript abundance in lesional skin and the VAS itch scores from patients with AD. R = 0.22 (CI = −0.13 to 0.52), P = 0.22. Error bars = standard error of the mean. AD, atopic dermatitis; CPM, counts per million; LS, lesional skin; NL, nonlesional; n.s., no significance; RNA-Seq, RNA Sequencing; VAS, visual analog scale.
Supplementary Figure S2. *Klk7* expression is restricted to the epidermis in mouse skin. (a) Genomic construct of the *Klk7*tm1(KOMP)Vlcg allele. Exons 3-5 within the coding region of *Klk7* are replaced with a LacZ-β-galactosidase reporter. (b) X-gal staining (blue) of hair-bearing skin from a *Klk7*LacZ mouse, in which LacZ expression is controlled under the *Klk7* promoter. (c–f) X-gal staining of (c) DRG, (d) TG, (e) SC, (f) and brain. (g) RT-PCR screening of *Klk7* expression in tissues from a WT control mouse. N = 3 biological replicates for panels (b–g). Scale bars = 200 µm. Cere., cerebrum; Cebel., cerebellum; DRG, dorsal root ganglia; Epidid., epididymis; Esoph., esophagus; G skin, glabrous skin; H skin, hairy skin; LacZ, β-galactosidase; RT-PCR, reverse transcriptase-PCR; SC, spinal cord; SI, Small intestine; TG, trigeminal ganglia; WT, wild-type.
Supplementary Figure S3. *Klk7*−/− mice do not show lowered Th2 and inflammatory markers after MC903 Treatment. (a, b) ELISA-based quantification of serum (a) IgE and (b) TARC levels in MC903-treated control and *Klk7*−/− mice. (c–e) RT-qPCR quantification of c. *Il4ra*, (d) *Il4*, and (e) *Il13* expression in MC903-treated skin of control and *Klk7*−/− mice. (f) RT-qPCR quantification of *Tslp* expression in vehicle or MC903-treated skin of control and *Klk7*−/− mice. Error bars = standard error of the mean. ***P < 0.001. MC903, calcipotriol; n.s., no significance, RT-qPCR, quantitative reverse transcriptase–PCR; TARC, thymus and activation-regulated chemokine.

Supplementary Figure S4. *Klk7*−/− mice have a selective reduction in itch in MC903 ear model. (a) Scratching behavior recorded on day 12 of MC903 treatment of control (wild-type) and *Klk7*−/− mice. (b) Change in ear thickness (normalized to pretreatment baseline) as measured by calipers daily during topical MC903 treatment. Error bars = standard error of the mean. **P < 0.05. WT, wild-type.

Supplementary Figure S5. rhKLK7 does not activate PAR2 receptors. Representative calcium transients of KRNK cells stably transduced with PAR2 expression after treatment with rhKLK7 and trypsin.
Supplementary Figure S6. Acute in vivo and in vitro effects of rhKLK7. (a, b). Acute pain (wiping) and itch (scratching) behavioral responses of C57BL/6J mice to vehicle or 1 µg rhKLK7 injections. (c) Quantification of calcium responses of culture DRG neurons from PirtGCaMP3+/+ mice to acute application of vehicle or 20 ng/µl rhKLK7. Percent responsive represents fraction of all DRG neurons in field. Data is presented as mean ± standard error of the mean. DRG, dorsal root ganglia; n.s, no significance.

Supplementary Table S1. Primers used for PCR

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Supplementary Table S2. Primary Antibodies for Flow Cytometry

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