TSLP Down-Regulates S100A7 and \( \beta \)-Defensin 2 Via the JAK2/STAT3-Dependent Mechanism

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Elevated T-helper type 2 cytokines in atopic skin, such as IL-4 and IL-13, were thought to be responsible for an impaired expression of antimicrobial proteins, which may contribute to the increased susceptibility to skin infections in patients with atopic dermatitis. In this study, the relationship between thymic stromal lymphopoietin and antimicrobial proteins and the involved molecular pathway was defined in normal human epidermal keratinocytes and human skin equivalent model. Stimulation of normal human epidermal keratinocytes with thymic stromal lymphopoietin decreased both mRNA and levels of S100A7 and human \( \beta \)-defensin 2 in a dose-dependent manner, and the regulation was JAK2/STAT3-dependent. Thymic stromal lymphopoietin decreased the antimicrobial protein expression, even in the presence of IL-17, which is their strong inducer. STAT3 directly regulated the S100A7 and human \( \beta \)-defensin 2 promoters in normal human epidermal keratinocytes. Immunohistochemically, lesional atopic skin stained more intensely with phospho-STAT3 compared with healthy control. Our results show that up-regulated thymic stromal lymphopoietin may contribute to the deficiency of antimicrobial proteins in atopic dermatitis, including S100A7 and human \( \beta \)-defensin 2, by a JAK2/STAT3-dependent mechanism and that STAT3/Sin3a might directly control the transcriptional activity of the antimicrobial protein promoters in normal human epidermal keratinocytes. Taken together, a key role of the JAK2/STAT3/Sin3a signaling pathway in thymic stromal lymphopoietin-mediated immune response in normal human epidermal keratinocytes might give us clues to understanding the pathological signal transductions in atopic dermatitis.


INTRODUCTION
Thymic stromal lymphopoietin (TSLP) is an IL-7-like cytokine leading to T-helper (Th) type 2 allergic inflammation and is highly expressed by keratinocytes in atopic dermatitis (AD) lesions (Soumelis et al., 2002). Skin-specific overexpression of TSLP induced AD-like phenotypes in mice, including a dramatic increase in \( CD4^+ \) Th2 cells expressing cutaneous homing receptors and elevated serum levels of IgE (Yoo et al., 2005). Keratinocytic TSLP could trigger atopic march in mice through skin sensitization to an allergen followed by a subsequent allergic asthma (Leyva-Castillo et al., 2013). TSLP receptor was recently found to be expressed in normal human epidermal keratinocytes (NHEKs), suggesting that NHEKs may be the major target of TSLP in the pathogenesis of AD (Kim et al., 2015; Kubo et al., 2014). An important issue that remains to be resolved is identification of the biological effects of TSLP on NHEKs.

The skin plays a major role in both innate and adaptive immune responses. Keratinocytes also synthesize various host defense molecules, including antimicrobial peptides (AMPs), with broad-spectrum activities against bacteria, fungi, and viral pathogens (Fulton et al., 1997; Gallo et al., 1994; Stolzenberg et al., 1997). AMPs act as effector molecules of innate immunity and bridge the innate and adaptive systems. It is well known that cutaneous infection is more prevalent in patients with AD. The expression of AMPs, such as cathelicidin (LL-37) and human \( \beta \)-defensin (hBD) 2, was significantly decreased in atopic lesions versus psoriatic lesions (Ong et al., 2002). Accordingly, the low levels of AMPs in patients with AD might contribute to the increased susceptibility to skin infection. AMP is inducible by disruption of the skin barrier and by proinflammatory cytokines like tumor necrosis factor-\( \alpha \), IL-1, IL-6, and IFN-\( \gamma \) (Erdag and Morgan, 2002; Liu et al., 2002; Nomura et al., 2003; Ong and Ohtake, 2002), which are strongly expressed in psoriasis skin (Grossman et al., 1989; Nomura et al., 2003). Well-known Th17
cytokines of IL-17 and IL-22 are also required for the expression of S100A7 and hBD2 (Liang et al., 2006). However, tumor necrosis factor-α and IFN-γ are decreased to negligible levels in AD skin (Nomura et al., 2003), and Th2 cytokines prevalent in AD, such as IL-4 and IL-13, inhibit AMP expression (Ong et al., 2002). Atopic lesions show high levels of Th2 cytokines and low levels of strong AMP inducers, including IL-1, IL-1, and IL-22, compared with psoriatic lesions (Pflander et al., 2010). The disparate cytokine milieu in AD and psoriasis might be responsible for the different AMP expression. However, it remains unknown whether TSLP affects AMP expression in the skin.

Signal transducer and activator of transcription 3 (STAT3) is a transcriptional factor involved in cell proliferation and differentiation (Akira et al., 1994; Fukada et al., 1996; Yamanaka et al., 1996). Increasing evidence suggests a role for STAT3 in allergic inflammation. STAT3 in the airway epithelium was implied as an important regulator of allergic inflammation in a murine model of asthma (Simeone-Penney et al., 2007), and inhibition of STAT3 signaling reduced degranulation of mast cells (Siegel et al., 2013). STAT3 also plays an essential role in the differentiation of Th17 cells to produce IL-17 (Yang et al., 2007). However, the expression and role of STAT3 and its regulator in the epidermis of AD is still not completely understood.

JAK2 is a member of the Janus kinase family and has been implicated in the signal transduction pathways of numerous cytokines and growth factors. Local JAK1 and JAK2 inhibition showed that targeting the JAK/STAT pathway might be an effective therapy in inflammatory skin diseases such as psoriasis and AD (Fridman et al., 2011). Topical application of JAK inhibitor resulted in suppression of STAT3 phosphorylation, edema, lymphocyte infiltration, and keratinocyte proliferation in a murine contact hypersensitivity model and inhibited tissue inflammation induced by either intradermal IL-23 or TSLP. In another study, topical JAK inhibitor successfully suppressed STAT3 activation and improved skin barrier function, increasing terminal differentiation proteins, such as filaggrin (Amano et al., 2015). A recent clinical study also showed the potential benefits of the oral JAK inhibitor (tofacitinib citrate) in the treatment of recalcitrant AD (Levy et al., 2015). We previously reported that TSLP down-regulates filaggrin expression by STAT3 and extracellular signal-regulated kinase phosphorylation in keratinocytes (Kim et al., 2015).

We hypothesized that TSLP could regulate AMP expression by direct control of STAT3 in NHEKs. Hence, we examined S100A7 and hBD2 expression in NHEKs and a human skin equivalent model (HSEM) after exposure to TSLP. We also investigated JAK2/STAT3 signaling as the potential pathway involved in the regulation of AMP expression by TSLP.

RESULTS

Down-regulation of S100A7 and hBD2 expression by TSLP in human keratinocytes

To identify whether TSLP affects AMP expression in the skin, NHEKs were treated with different doses (0, 1, 10, and 100 ng/ml) of TSLP for 24 hours. Both mRNA expression and protein levels of S100A7 and hBD2 mRNA were decreased in a dose-dependent manner (Figure 1a and b). A luciferase assay to evaluate whether TSLP affects the activity of AMP promoter showed that TSLP reduced the luciferase activities of S100A7 and hBD2 promoters (Figure 1c). We used HSEMs to confirm the effects of TSLP on S100A7 and hBD2 expression in NHEKs. HSEMs are multilayered, three-dimensional tissue models that are very similar to the normal human epidermis. After stimulation with TSLP (500 ng/ml) for 24 hours, immunohistochemical analysis showed decreased S100A7 and hBD2 expressions in TSLP-stimulated HSEMs compared with those in untreated HSEMs (Figure 1d). After treatment with low-dose (100 ng/ml) TSLP, S100A7 and hBD2 expression were also decreased in TSLP-stimulated HSEMs compared with controls (see Supplementary Figure S1 online). Moreover, we investigated the relative contribution of each IL-4, IL-13, IL-33, and TSLP in suppressing AMP expression in keratinocytes. This study suggested that TSLP might be one of the major contributors to down-regulating S100A7 and hBD2 expression in keratinocytes (see Supplementary Figure S2 online). It is well known that other AMPs such as hBD3, RNase 7, and LL37 are very important for the control of Staphylococcus aureus growth. Therefore, we investigated the effect of TSLP on the induction of hBD3, RNase 7, and LL37. Our results showed that the levels of hBD3 mRNA were significantly down-regulated after exposure to TSLP in human keratinocytes (see Supplementary Figure S3a online). In addition, levels of hBD3 protein were also decreased after TSLP treatments (see Supplementary Figure S3b).

Suppression of IL-17A-induced S100A7 and hBD2 expression by TSLP

Because Th17 cells are increased in AD patients with enhanced IL-17 expression, we showed that stimulation of NHEKs with IL-17 resulted in an increased antimicrobial protein S100A7 and hBD2 expression, a phenomenon that has been described (Koga et al., 2008; Liang et al., 2006). In contrast, AMPs were shown to be decreased in acute and chronic lesions from patients suffering from AD (Hata and Gallo, 2008). To explain the discrepancy, we investigated the inhibition of S100A7 and hBD2 expression in NHEKs by TSLP. Levels of S100A7 and hBD2 mRNA and protein were diminished in IL-17A and TSLP-stimulated NHEKs compared with those in IL-17A–treated NHEKs (see Supplementary Figure S4a and b online). We confirmed this finding by immunohistochemical analysis of HSEM (see Supplementary Figure S4c).

Regulation of S100A7 and hBD2 expression by TSLP via the JAK2/STAT3 signaling pathways

To identify the determinants of S100A7 and hBD2 expression in NHEKs, the promoter regions of the S100A7 and hBD2 genes were analyzed. The expression of candidate transcription factors in NHEKs was evaluated after TSLP treatment. TSLP induced a STAT3 expression in keratinocytes but not AMP response element-binding protein or NF-κB (see Supplementary Figure S5 online), implying that STAT3 might be a major regulator of S100A7 and hBD2 expression. Previously, an effective suppression of STAT3 phosphorylation by JAK inhibitors was reported, and we have shown before that TSLP inhibits filaggrin expression via STAT3 in keratinocytes (Amano et al., 2015; Fridman et al., 2011; Kim et al., 2015).
To investigate the involvement of JAK1, JAK2, and STAT3 in TSLP-induced down-regulation of S100A7 and hBD2, we evaluated JAK1, JAK2, and STAT3 protein levels in TSLP-treated keratinocytes. Figure 2a shows that TSLP (100 ng/ml) significantly increased levels of phosphorylated JAK2 and STAT3 proteins, indicating that TSLP may activate the JAK2 and STAT3 pathways. Next, we examined the effects of blocking different signaling pathways with specific inhibitors to define which one is necessary for TSLP-mediated inhibition of S100A7 and hBD2 expression. As shown in Figure 2b, blocking JAK2 activation with the specific JAK inhibitor pyridone 6 abolished the effect of TSLP for AMP expression, which restored the expression of S100A7 and hBD2 protein in TSLP-treated keratinocytes. These findings suggested that TSLP-induced STAT3 expression might be mediated via the JAK2 pathway in NHEKs. In addition, the expression and phosphorylation of the mitogen-activated protein kinase proteins were investigated in TSLP-treated keratinocytes. Extracellular signal-regulated kinase and c-Jun N-terminal kinase phosphorylation increased after exposure to TSLP (see Supplementary Figure S6a online). However, blocking mitogen-activated protein kinase activation with specific mitogen-activated protein kinase inhibitors (U0126, SB239063, and SP600125) could not recover TSLP-suppressed S100A7 and hBD2 expression (see Supplementary Figure S6b), showing that S100A7 and hBD2 expression were not controlled via the mitogen-activated protein kinase signaling cascade.

To confirm the role of STAT3 in S100A7 and hBD2 expression, we used a constitutively activated STAT3 system. Levels of S100A7 and hBD2 protein and promoter activities were examined after transfection with pcDNA3.1/STAT3 or no insert in keratinocytes. Both the expression of S100A7 and hBD2 protein and the transcriptional activities of S100A7 and hBD2 promoters were decreased by transfection with pcDNA3.1/STAT3 (Figure 2c and d). To further determine the dependency on the STAT3 signaling pathway, we analyzed the effects of the STAT3 inhibitor STA-21 and small interfering RNA (siRNA) on the expression of S100A7 and hBD2 protein after exposure to TSLP. The TSLP-induced decrease in levels of S100A7 and hBD2 protein was restored by STAT3 siRNA and STA-21 (Figure 2e and f). Immunofluorescence similarly showed that STA-21 reinstated the TSLP-suppressed S100A7 and hBD2 expression (see Supplementary Figure S7 online). These data suggest that TSLP can regulate S100A7 and hBD2 expression through a JAK2/STAT3-dependent mechanism.
Up-regulated STAT3 expression in lesional atopic skin
To our knowledge, the expression of STAT3 in atopic skin lesions has never been studied. To investigate if STAT3 is related to the atopic phenotype, we performed immunohistochemical staining using a specific phospho-STAT3 antibody in skin biopsy samples from patients with AD and normal healthy volunteers. 

STAT3 was highly expressed in atopic lesional skin compared with normal control skin (Figure 3). These results suggest that STAT3 may be involved in the pathogenesis of AD.

Control of transcriptional activities of the S100A7 and hBD2 promoters through STAT3 and Sin3a
A site-directed mutagenesis analysis was performed to investigate whether STAT3 suppresses S100A7 and hBD2 expression by binding directly to their promoters (see Supplementary Figure S8 online). Site-directed mutagenesis of the putative STAT3 sequences in the S100A7 and hBD2 promoters prevented their activation after TSLP treatment with or without STAT3 overexpression (see Supplementary Figure S9 online). Whether STAT3 suppresses AMP expression by direct binding to their promoters was evaluated by electrophoretic mobility shift assays performed in TSLP-treated keratinocytes. Figure 4b shows that STAT3 binding to the S100A7 and hBD2 promoter increased after incubating nuclear extracts from TSLP-treated keratinocytes with the STAT3 oligonucleotide (elements 1–3), and a supershifted band was noted after adding an anti-pSTAT3-specific antibody to the nuclear extracts. Competition for STAT3 binding with unlabeled oligonucleotides containing STAT3 (elements 1–3) abolished the STAT3-shifted bands.

In contrast, other reports (Hau et al., 2013; Kanda and Watanabe, 2008) show that STAT3 activation by various stimuli can mediate the induction of S100A7 and hBD2. To explain this difference, we hypothesized the epigenetic regulation of STAT3 expression. A previous study (Icardi et al., 2012) presented the SIN3 transcription regulator homolog A (Sin3a) as a major regulator of STAT3-targeted transcriptional repression. Sin3a may directly interact with STAT3 and promote its deacetylation.

We found that levels of Sin3a mRNA and protein were increased after TSLP treatment in keratinocytes (see Supplementary Figure S10 online). In addition, treatment with Sin3a siRNA resulted in the restoration of S100A7 and hBD2 expression in human keratinocytes (Figure 4c).
further verify our assumption that Sin3a directly binds and controls STAT3 binding sites of S100A7 and hBD2 promoters, we performed chromatin immunoprecipitation assays using an anti-Sin3a specific antibody. To rule out the nonspecific binding of antibodies, unspecific anti-IgG antibody was used as a negative control. This study showed that STAT3 and Sin3a could bind to the S100A7 and hBD2 promoters (Figure 4d). This study shows that Sin3a may be an important mediator of STAT3 transcriptional repression in the regulation of S100A7 and hBD2 expression by TSLP.

DISCUSSION
The skin is the front line against external environmental attacks with potential microbial pathogens. Although the skin was formerly considered an inactive physical protective barrier participating in host immune defense merely by blocking entry of pathogens, it is now apparent that the skin defends the body by rapidly mounting an innate immune response to injuries and microbial insults (Braff and Gallo, 2006). The primary defense by the cutaneous innate immune mechanisms is endogenous AMPs secreted by keratinocytes, including hBD2 (Fulton et al., 1997; Harder et al., 1997), LL-37 (Frohm et al., 1997), Rnase7, and S100A7 (psoriasin). hBD2 and S100A7 are typical markers of alterations in skin barrier function in atopic dermatitis (Onderdijk et al., 2015). Defensins are cationic peptides containing cysteine-rich conserved motifs with a broad antibacterial activity against gram-positive and -negative bacteria, fungi, and viruses (Hata and Gallo, 2008). Of the four HBDs, unlike constitutively expressed hBD1, hBD2 and 3 are inducible by bacterial infection; cytokines IL-1α, IL-1β, and tumor necrosis factor-α; and differentiation (Liu et al., 2002). S100 proteins have been receiving attention as key players in innate immunity, important in the pathogenesis of various inflammatory, metabolic, and neoplastic disorders. First identified as overexpressed in psoriatic scales, S100A7 is now known to potentiate immune-mediated inflammatory processes in the skin (Madsen et al., 1991). It is a chemottractant for various leukocyte subsets, bridging innate and adaptive immunity (Wolf et al., 2008). S100A7 also acts as an alarm to prime keratinocytes and neutrophils for enhanced production of proinflammatory cytokines, favoring microbialidal capacities (Hegyi et al., 2012). Similar to hBD2, it is inducible by Th1-, Th17-, and Th22-derived cytokines; inflammation; and exposure to microorganisms, suggesting its involvement in cutaneous defense and inflammation (Glaser et al., 2005). A reduced induction of AMPs in conjunction with defects in the epidermal barrier in AD may contribute to the increased susceptibility of AD skin to S. aureus infection.
Basal levels of AMP expression on the skin of normal healthy humans are not fully established. However, some researchers have reported that S100A7 and hBD2 were constitutively expressed in healthy human skin (Chiricozzi et al., 2014; Kesting et al., 2010) and human skin equivalent models (Glaser et al., 2005). Further studies are needed to elucidate the exact levels of AMP expression in normal healthy skin. Atopic skin has been thought to be characterized by an impaired induction of AMPs, which might be ascribable to elevated levels of Th2 cytokines in AD, such as IL-4, IL-10, and IL-13 (Nomura et al., 2003; Ong et al., 2002). The principal Th2 cytokine IL-4 was reported to down-regulate AMPs or factors involved in AMP production; hBD2, S100s, IFN-κ, Toll-like receptors, and several chemokines were down-regulated by IL-4 in keratinocytes (Albanesi et al., 2007; Bao et al., 2013; Ong et al., 2002). IL-33, which is another Th2 cytokine crucial in AD, showed the capacity to down-regulate hBD2 in keratinocytes, to a lesser extent than IL-4 (Alase et al., 2012). Neutralizing IL-10 in AD skin explants with anti–IL-10 augmented the expression of both hBD-2 and LL-37 (Howell et al., 2005). It seems that various cytokines could differentially control AMP expression (Alase et al., 2012; Min et al., 2014).

We looked into TSLP and its main source, keratinocytes, because TSLP is considered the major initiating player in immunopathogenesis of AD (Soumelis et al., 2002; Ying et al., 2008). TSLP is an IL-7–like cytokine promoting type 2 cytokine-mediated inflammations, including AD, allergic rhinitis, and asthma (Ziegler, 2012). Keratinocytic TSLP triggered the atopic march in mice, and mice overexpressing TSLP in the skin showed AD with eczematous lesions, higher circulating Th2 cell population, and increased serum IgE levels (Leyva-Castillo et al., 2013; Yoo et al., 2005). Despite the evidence that TSLP is highly expressed in acute and chronic AD lesions mainly by keratinocytes in human skin, the effects of TSLP on human keratinocytes are still not completely elucidated.

Two TSLP isoforms (short and long) have been reported in human subjects. The isoforms have been reported to exhibit different activities (Bjerkan et al., 2015; Fornasa et al., 2015). The short isoform has anti-inflammatory activities. In contrast, the long isoform of TSLP is proinflammatory and is only induced during inflammation, such as in the lesional skin of AD (Fornasa et al., 2015). In this study, the long form of TSLP has been used to evaluate the role of TSLP in inflammatory disorders.
We hypothesized that the down-regulation of AMPs in AD would be facilitated by another type 2 cytokine, TSLP, aggravating AD. In this study, we verified that TSLP reduced mRNA and protein expressions of S100A7 and hBD2 in a dose-dependent manner, using NHEKs and HSEMs. Impaired AMP induction by Th2 cytokines could be the result of a Th2-mediated defective Th17 system (Wolk et al., 2014). Consistent with this, TSLP suppressed the IL-17A–induced S100A7 and hBD2 production. As for the responsible signaling cascade, the JAK2/STAT3 pathway was evaluated. STAT3 is a transcriptional factor that regulates various immune and inflammatory responses (Levy and Darnell, 2002; Simeone-Penney et al., 2007). Strengthening of allergic inflammation by STAT3 was shown in studies where disruption of epithelial STAT3 reduced airway eosinophilia, and IL-17A–induced STAT3 activation mediated eotaxin-1/CCL11 induction in human airway smooth muscle cells (Saleh et al., 2009). Hyper-IgE syndrome, known to be caused by mutated STAT3 genes, may show AD-like skin lesions. Although TSLP increases the phosphorylation of STAT3, TSLP-induced Sin3a can modulate the transcriptional activity of STAT3 in keratinocytes. In addition, a recent cohort study showed that allergic diseases such as food allergies and anaphylaxis were decreased in patients with hyper-IgE syndrome compared with patients with no STAT3 mutation (Siegel et al., 2013). Further studies are needed to better understand the role and regulatory mechanism of STAT3 in hyper-IgE syndrome.

JAK was shown to be associated with the regulation of STAT expression in cells exposed to IL-4 or TSLP (Rochman et al., 2010). JAK1 and JAK2 inhibition suppressed STAT3 stimulation and decreased TSLP-caused tissue inflammation (Amano et al., 2015; Fridman et al., 2011). We found that TSLP significantly induced phosphorylated JAK2 and STAT3 proteins, indicating that TSLP might activate the JAK2/STAT3 pathway. Interrupting JAK2 activation with specific JAK inhibitor and STAT3 blockade with the STAT3 inhibitor and siRNA restored the expression of S100A7 and hBD2 protein in keratinocytes, which was decreased by TSLP treatment. However, blocking mitogen-activated protein kinase, including extracellular signal-regulated kinase and c-Jun N-terminal kinase, failed to recover TSLP-induced down-regulation of S100A7 and hBD2. In addition, immunohistochemical study showed that pSTAT3 was highly expressed in atopic lesional skin compared with normal control skin. Taken together, these results suggest that STAT3 and JAK2 assist with the control of AMP expression by TSLP in keratinocytes.

This study explains a key role of the JAK2/STAT3/Sin3a signaling pathway in TSLP-mediated immune response in human keratinocytes. We link S100A7 and hBD2 expression with TSLP stimulation and report that TSLP down-regulates the production of S100A7 and hBD2 by keratinocytes via the JAK2/STAT3/Sin3a-dependent pathway. Considering up-regulation of TSLP and STAT3 in patients with AD, these results may give us valuable clues to understanding the pathological signal transductions in AD.

**MATERIALS AND METHODS**

**DNA construction**

The S100A7 promoter (−1080/+1 base pairs) linked to the luciferase gene was cloned by PCR from human genomic DNA for the S100A7 promoter assay using the following primers: 5′-GGCGTACCGTCTGCCCCAAGTG-3′ and 5′-CGGAAGCTTGCAGGGCCGGGAACA-3′. After PCR amplification, the fragment was digested with KpnI/HindIII and then ligated into the pGL3.0 luciferase reporter vector, and the hBD2 promoter (−1545/+1 base pairs) linked to the luciferase gene was cloned by PCR from the human genomic DNA with the following primers: 5′-AGCTTTGCTCTGGAAGG-3′ and 5′-GAGTCTGGGAGGACATCAA-3′. After PCR amplification, the fragment was digested with XhoI/HindIII and then ligated into the pGL3.0 luciferase reporter vector (Promega, Madison, WI). Sequencing and orientation of the insert were verified by sequencing analysis.

The pcDNA3.1/STAT3 expression vector containing pcDNA3.1/STAT3 fused with green fluorescent protein or the empty vector were transfected into keratinocytes using the Lipofectamine Plus reagent (Invitrogen, Carlsbad, CA) to ectopically express pcDNA3.1/STAT3.

**Gene silencing**

STAT3 siRNA and Sin3a siRNA (GenePharma Co., Shanghai, China) were used for STAT3 and Sin3a gene silencing. Scrambled siRNA was used as control siRNA. Cells that were approximately 40% confluent in 60-mm cell culture dishes were transfected with 20 nmol/L siRNA using lipofectamine according to the manufacturer's instructions. The cells were allowed to stabilize for 24 hours before being used in experiments.

**Electrophoretic mobility shift assay**

The electrophoretic mobility shift assay was performed using the LightShift Chemiluminescent RNA EMSA assay kit (ThermoScientific, Rockford, IL) according to the manufacturer's instructions. Nuclear extracts were obtained from TSLP-treated keratinocytes. Biotin-conjugated oligonucleotides containing the wild-type STAT3 sequence (S100A7 element-1, AGACTTTCTGGGAAAGTTA; S100A7 element-2, CTCCTTCTTTAAATAGA; S100A7 element-3, AGCCTTCTGGGAAAGTTA; hBD2 element-1, GGTTTCTCCGAACCCTGA; hBD2 element-2, TGTGTTCACTGCCCT; hBD2 element-3, ACTTTCCATATAATTCTA) were used. Excessive amounts of unlabeled competitors were added 5 minutes before adding the labeled probes for the competition assays. A pSTAT3 antibody (Cell Signaling Technology, Danvers, MA) (1 μg) was added and incubated at 4 °C for 60 minutes in the supershift assay.

**Chromatin immunoprecipitation assay**

Cells were incubated for 24 hours after treatment with TSLP and then treated with 1% formalin for 10 minutes at 37 °C. The cells were harvested, and chromatin immunoprecipitation was performed using a chromatin immunoprecipitation assay kit (Upstate Biotechnology, Temecula, CA), according to the manufacturer's instructions. After immunoprecipitation, the captured genomic fragments were recovered by phenol-chloroform extraction. The S100A7 and hBD2 promoter fragments were identified by PCR analysis using promoter-specific primers. PCR was performed for 35 cycles, and the amplified products were analyzed on a 2% agarose gel. The oligonucleotide sequences used for PCR amplification of the S100A7 and hBD2 promoters were as follows: S100A7 element-1, 5′-AAAA CACTGAAAGACTTCTGGGAA-3′ (forward) 5′-AGTGTGACTGCTG-3′ (reverse); S100A7 element-2, 5′-TGGAACATCT CAACACTTCC-3′ (forward) 5′-CAGCGTGACTGAGATT-3′ (reverse); S100A7 element-3, 5′-GTCTTTCACTGGCAACCAACAACAC-3′ (forward) 5′-GGACTTTCAAGAAAAAGCAGAA-3′ (reverse); hBD2 element-1, 5′-CTGCCAGGAATCGGTCTC-3′ (forward) 5′-CTGTG...
Supplementary material is linked to the online version of the paper at www.jidonline.org, and at http://dx.doi.org/10.1016/j.jid.2016.07.027.

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CONFLICT OF INTEREST

The authors state no conflict of interest.


