



# Transglutaminase 3 Promotes Skin Inflammation in Atopic Dermatitis by Activating Monocyte-Derived Dendritic Cells via DC-SIGN

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Atopic dermatitis (AD) is often concomitant with increased levels of IgE against not only foreign allergens but also autoallergens. AD patients with autoallergy are likely to be more severe and difficult to treat, and self-reactive IgE might be a contributing factor in the pathogenesis of AD. However, how autoallergens are recognized by the immune system and what immune responses are induced subsequently remain largely unknown. We found that the serum level of IgE against transglutaminase 3 (TGase3) was significantly higher in AD patients than in healthy individuals and was positively correlated with disease severity. The expression of TGase3 in the lesional skin of AD patients was markedly increased compared with that of the controls, and Th2 cytokines and/or allergen promoted the expression of TGase3 in keratinocytes. TGase3 bound monocyte-derived dendritic cells (MoDCs) via dendritic cell-specific ICAM-3-grabbing non-integrin (DC-SIGN), which resulted in the production of IL-6 and activation of the NF- $\kappa$ B signaling pathway in MoDCs; and TGase3-treated MoDCs facilitated Th1 polarization. Moreover, skin inflammation in the mouse model of MC903-induced AD was attenuated when TGase3 was inhibited. In conclusion, TGase3 was revealed as an autoallergen in AD and actively involved in skin inflammation; TGase3-targeting might be a therapeutic strategy for the treatment of AD.

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## INTRODUCTION

Atopic dermatitis (AD) is a common chronic relapsing skin disorder with multifactorial causes, and more than 80% of AD patients have increased levels of total and/or specific IgE

against foreign allergens (Kim et al., 2016). Studies have also shown that there is a high prevalence of IgE against autoantigens (Zeller et al., 2009). Autoallergen-induced cytokines and T cell responses contribute to the exacerbation and chronicity of the inflammation in AD (Valenta et al., 2000). Until now, more than 140 human autoantigens have been identified in AD patients as potential candidates for IgE-binding, which include manganese superoxide dismutase (MnSOD), P<sub>2</sub> ribosomal protein, and cyclophilin (Flückiger et al., 2002a; Flückiger et al., 2002b; Mayer et al., 1999). Many of the autoallergens are cross-reactive with exogenous allergens. For example, MnSOD shares a homologous sequence with an allergen from *Aspergillus fumigatus* and is supposed to induce immune responses similar to that of exogenous allergens (Schmid-Grendelmeier et al., 2005). Other autoallergens in AD lack homology to known environmental allergens and are also involved in skin inflammation of AD; for example, Hom s1 and Hom s2 induce a Th1-dominated response (Heratizadeh et al., 2011; Valenta et al., 1998). However, how autoallergens are recognized by the immune system and what immune responses autoallergens elicit in AD remain largely unknown.

Transglutaminase 3 (TGase3), a member of the transglutaminases family, is expressed in the upper epidermal layers (Ahvazi et al., 2004; Tatsukawa et al., 2015) and shares a homologous sequence with *Dermatophagoides farinae* (Derf), one of the most prevalent environmental allergens (Ichikawa et al., 1998). The principal function of TGase3 is to cross-link a number of structural proteins to form a cell envelope (Candi et al., 1999; Melino and Piacentini, 1998; Steinert et al., 1999) and contribute to the functionally

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Abbreviations: Ab, antibody; AD, Atopic dermatitis; BCA, Bicinchoninic acid; CBA, Cytometric Bead Array; CLR, C-type lectin receptor; DC, dendritic cell; DC-SIGN, dendritic cell-specific ICAM-3-grabbing non-integrin; FCM, flow cytometry; HRP, horseradish peroxidase; IL, interleukin; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LPS, lipopolysaccharide; MnSOD, manganese superoxide dismutase; MoDC, monocytes-derived dendritic cell; MFI, mean fluorescence intensity; NF- $\kappa$ B, nuclear factor kappa-light-chain-enhancer of activated B cells; OD, optical density; qRT-PCR, quantitative real-time RT-PCR; SCORAD, Scoring Atopic Dermatitis Index; siRNA, small interfering RNA; TGase3, transglutaminase 3; TNF, tumour necrosis factor

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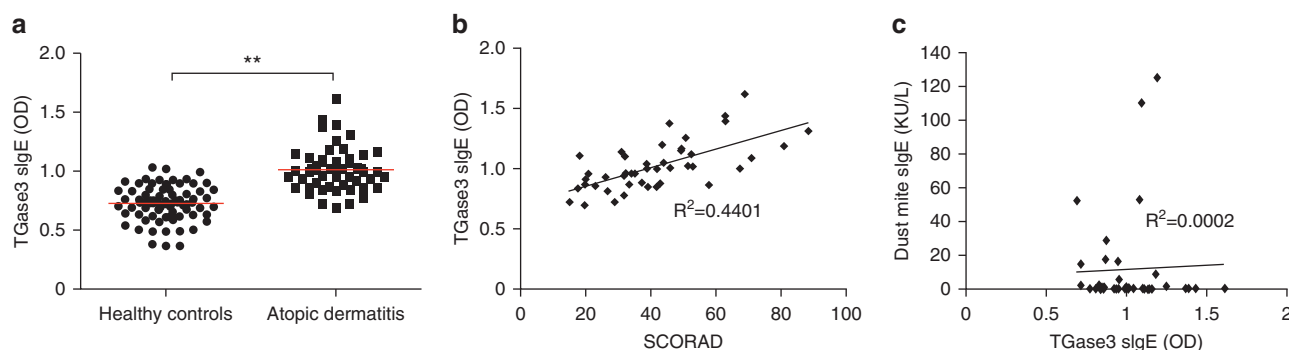


**Figure 1. TGase3-specific IgE in sera of AD patients.** (a) Mean optical density (OD) value of TGase3-specific IgE in sera of AD patients and healthy controls. AD,  $n = 46$ ; healthy controls,  $n = 59$ . \* $P < 0.05$ , \*\* $P < 0.01$ . (b) Correlation analysis for TGase3-specific IgE and SCORAD of AD patients. (c) Correlation analysis for TGase3-specific IgE and dust mite-specific IgE of AD patients. AD, atopic dermatitis; OD, optical density; SCORAD, Scoring Atopic Dermatitis Index.

intact cutaneous barrier (Bognar et al., 2014). The gene of *TGase3* locates in the genomic regions that are linked to AD (Bradley et al., 2002). The expression of *TGase3* is markedly upregulated in a variety of skin inflammations, including AD and UV radiation (Frezza et al., 2017), and *TGase3* has been recognized as an autoantigen that participates in the pathogenesis of dermatitis herpatiformis (Sárdy et al., 2002). Moreover, *TGase3* also has other functions that are not related to protein cross-linking, such as being a stress protein. However, the exact role of *TGase3* in the pathogenesis of AD is not clear.

Considering the homology between *TGase3* and Derf and the increased expression of *TGase3* in the skin under inflammatory conditions, we hypothesized that *TGase3* might act as an autoallergen and be involved in the skin inflammation of AD. In this study, we found that the level of *TGase3*-specific IgE was significantly increased in the sera of AD patients compared to that of healthy individuals, and the expression of *TGase3* was elevated in the lesional skin of AD. *TGase3* activated the NF- $\kappa$ B signaling pathway in monocyte-derived dendritic cells (MoDCs) through binding of dendritic cell-specific ICAM-3-grabbing non-integrin (DC-SIGN) and induced Th1 polarization. *TGase3* was revealed as an autoallergen in AD, which plays a pro-inflammatory role in the pathogenesis of AD.

## RESULTS

### Elevated level of TGase3-specific IgE in AD patients

We screened the specific IgE against dust mite and two self-antigens, *TGase3* and tubulin, that share a homologous sequence with Derf (Eriksson et al., 2001; Saarne et al., 2003) in the sera of 46 AD patients and 59 healthy controls using Capture ELISA. The result showed that there was a much higher level of *TGase3*-specific IgE in the sera of AD patients than healthy controls (Figure 1a). The patients were then stratified by age and disease severity, and there was no difference in the serum level of *TGase3*-specific IgE among the three age groups (12–18 years old, 19–40 years old, and 41–65 years old, Supplementary Figure 1a), while *TGase3*-specific IgE was positively correlated with the Scoring Atopic Dermatitis Index of AD patients ( $R^2 = 0.4401$ ,  $P < 0.05$ ) (Figure 1b, Supplementary Figure 1b). However, there was no difference in the level of tubulin-specific IgE between AD

patients and healthy controls (data not shown). The level of serum IgE against dust mite was also increased in AD patients; however, there was no correlation between *TGase3*-specific IgE and that against dust mite ( $R^2 = 0.0002$ ,  $P > 0.05$ ) (Figure 1c). The specifically increased autoreactivity to *TGase3* in AD patients indicated that *TGase3* might be involved in the pathogenesis of AD.

### Increased expression of TGase3 in the lesional skin of AD patients

We next analyzed the expression of *TGase3* in the skin lesions of AD patients. The result of confocal microscopy showed that *TGase3* was mainly located in the stratum corneum of normal skin and in AD lesion *TGase3* located in upper granular and cornified layers, and the staining was much higher than that in normal skin (Figure 2a). The epidermis was then separated from skin biopsies and subjected for quantitative real-time RT-PCR (qRT-PCR) and western blotting analysis. The results of qRT-PCR showed that the relative fluorescence intensity of *TGase3* was significantly higher in AD patients than in healthy controls (Figure 2b), which was consistent with the result of western blotting (Figure 2c). As *TGase3* is a stress-responsive protein, and the expression of *TGase3* is upregulated under various stress conditions, we analyzed the expression of *TGase3* in primary keratinocytes under the stimulation with pro-inflammatory cytokines and/or allergens. The result showed that the expression of *TGase3* was significantly increased at both the mRNA and protein levels in keratinocytes upon stimulation with IL-1 $\beta$ , IL-4, Derp2, or IL-4 plus Derp2 (Figure 2d–f). The increased expression of *TGase3* in the lesional skin of AD patients indicated that *TGase3* might be involved in the skin inflammation of AD.

### Recognition of TGase3 by MoDCs via DC-SIGN

We set out to explore how *TGase3* is recognized by the immune system. DCs in the skin are characterized by the expression of a high level of C-type lectin receptors (CLRs) that not only recognize foreign antigen but also autoantigens (García-Vallejo et al., 2014). We assessed the binding of *TGase3* by DC-SIGN and Langerin, the two most important CLRs in DCs of the skin. We first analyzed the expression of N-linked glycans in *TGase3* by liquid chromatography-tandem mass spectrometry (LC-MS/MS). One that the N-

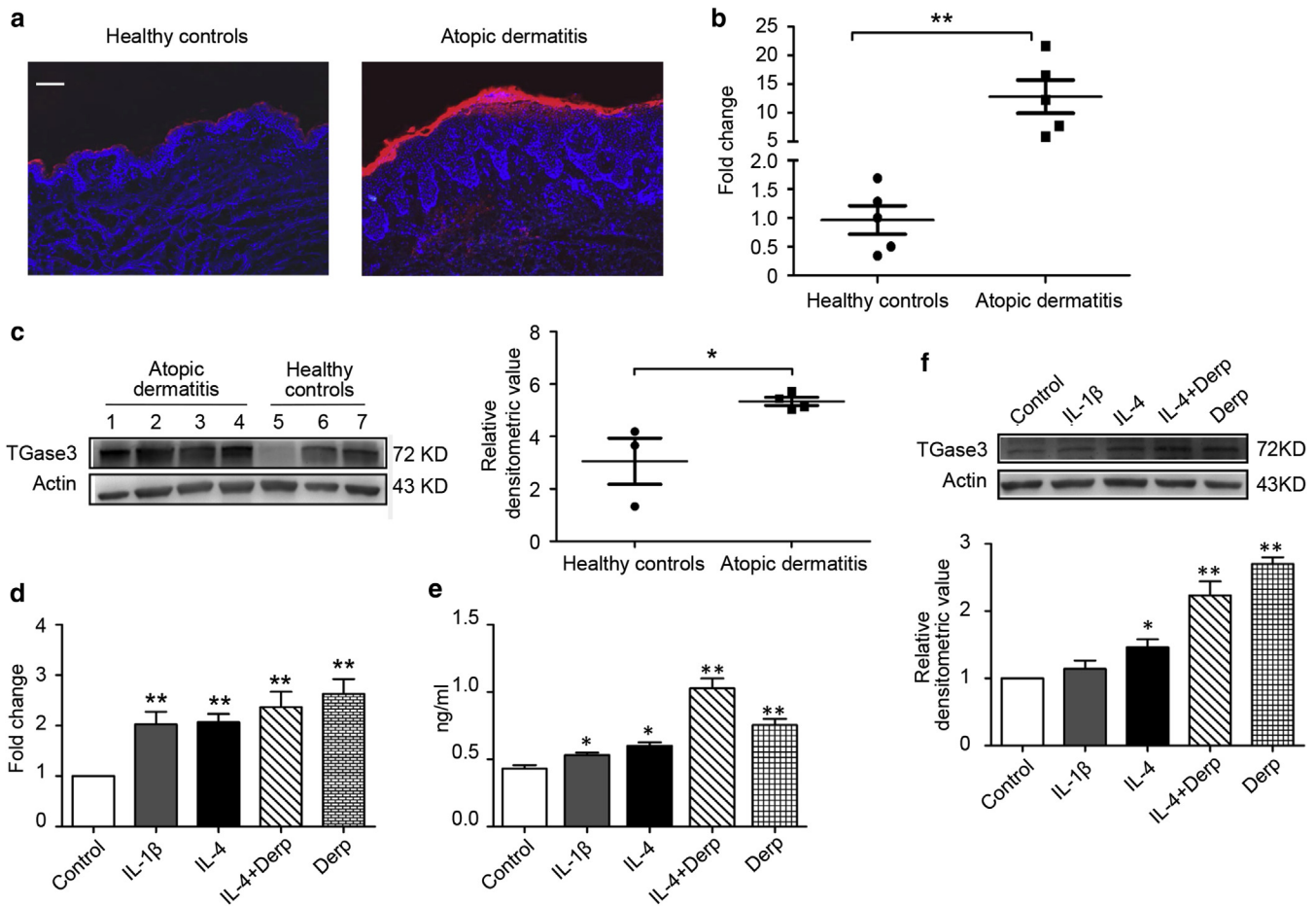


**Figure 2. Expression of TGase3 in the lesional skin of AD patients and keratinocytes.** (a) Immunofluorescence staining of TGase3 in lesional skin of AD patients and healthy controls. Original magnification: 200X. Scale bar = 100  $\mu$ m. Data are representative of at least five independent experiments. (b and c) qRT-PCR (b) and Western blotting (c) showing the expression of TGase3 in the epidermis of AD and healthy controls. Densitometry quantifies the expression levels of TGase3 relative to  $\beta$ -actin. Data are shown as mean  $\pm$  standard deviation. AD, n = 6; healthy controls, n = 9. \* $P$  < 0.05, \*\* $P$  < 0.01. (d-f) qRT-PCR (d), ELISA (e) and western blotting (f) showing the expression of TGase3 in primary keratinocytes stimulated with IL-1 $\beta$ , IL-4, Derp2, or IL-4 plus Derp2. Densitometry quantifies the expression levels of TGase3 relative to  $\beta$ -actin. Data are shown as the mean  $\pm$  standard deviation and are representatives of at least three independent experiments. \* $P$  < 0.05, \*\* $P$  < 0.01. AD, atopic dermatitis; qRT-PCR, quantitative real-time RT-PCR.

glycosylation site was found at position 404 (Supplementary Figure 2), which indicated that TGase3 could be a ligand for CLRs. Next, we assessed the binding of TGase3 to DC-SIGN or Langerin using solid phase ELISA, in which TGase3 was used as coating antigen. The result showed that TGase3 bound recombinant soluble DC-SIGN, but not Langerin, with variable binding abilities (Figure 3a). The binding of TGase3 to DC-SIGN was  $\text{Ca}^{2+}$ -dependent, as the binding was abrogated by the addition of 10 mM EDTA (data not shown). MoDCs had been shown to highly express DC-SIGN (Zhang et al., 2016) and were also used to explore the binding of TGase3 by DC-SIGN. The flow cytometry (FCM) result showed that there was increased staining of TGase3-Alexa Fluor 647 on MoDCs compared with the staining with the fluorescent dye control (Figure 3b). The immunofluorescence result showed that Alexa Fluor 647-labeled TGase3 co-localized with Alexa Fluor 488-anti-DC-SIGN antibody on MoDCs, indicating that TGase3 bound DC-SIGN on the surface of MoDCs (Figure 3c). When DC-SIGN-blocking Abs was added during the incubation, the binding of TGase3 to MoDCs was attenuated, indicating that

the binding was DC-SIGN dependant (Figure 3b and c). When the expression of DC-SIGN was knocked-down using DC-SIGN-specific siRNA, there was a significant reduction in mean fluorescence intensity (MFI) of TGase3-Alexa Fluor 647 on Mo-DCs, as shown in Figure 3d and e. Collectively, these data indicated that TGase3 could be recognized by DCs via DC-SIGN.

#### Activation of MoDCs by TGase3

To explore the function of TGase3 in the skin inflammation of AD, we assessed the effects of TGase3 on the activation of MoDCs. MoDCs from healthy individuals were incubated with TGase3 at 10  $\mu$ g/ml for 24 hours and subjected for the analysis of cytokine production, co-stimulation molecule expression and NF- $\kappa$ B signaling activation. The results of Cytometric Bead Array (CBA) for human inflammatory cytokines showed that the level of IL-6 was significantly increased in the supernatant of MoDCs culture under the stimulation with TGase3 compared with that from MoDCs cultured in the control medium (Figure 4a). The addition of anti-DC-SIGN blocking Abs at 5  $\mu$ g/ml inhibited the TGase3-induced

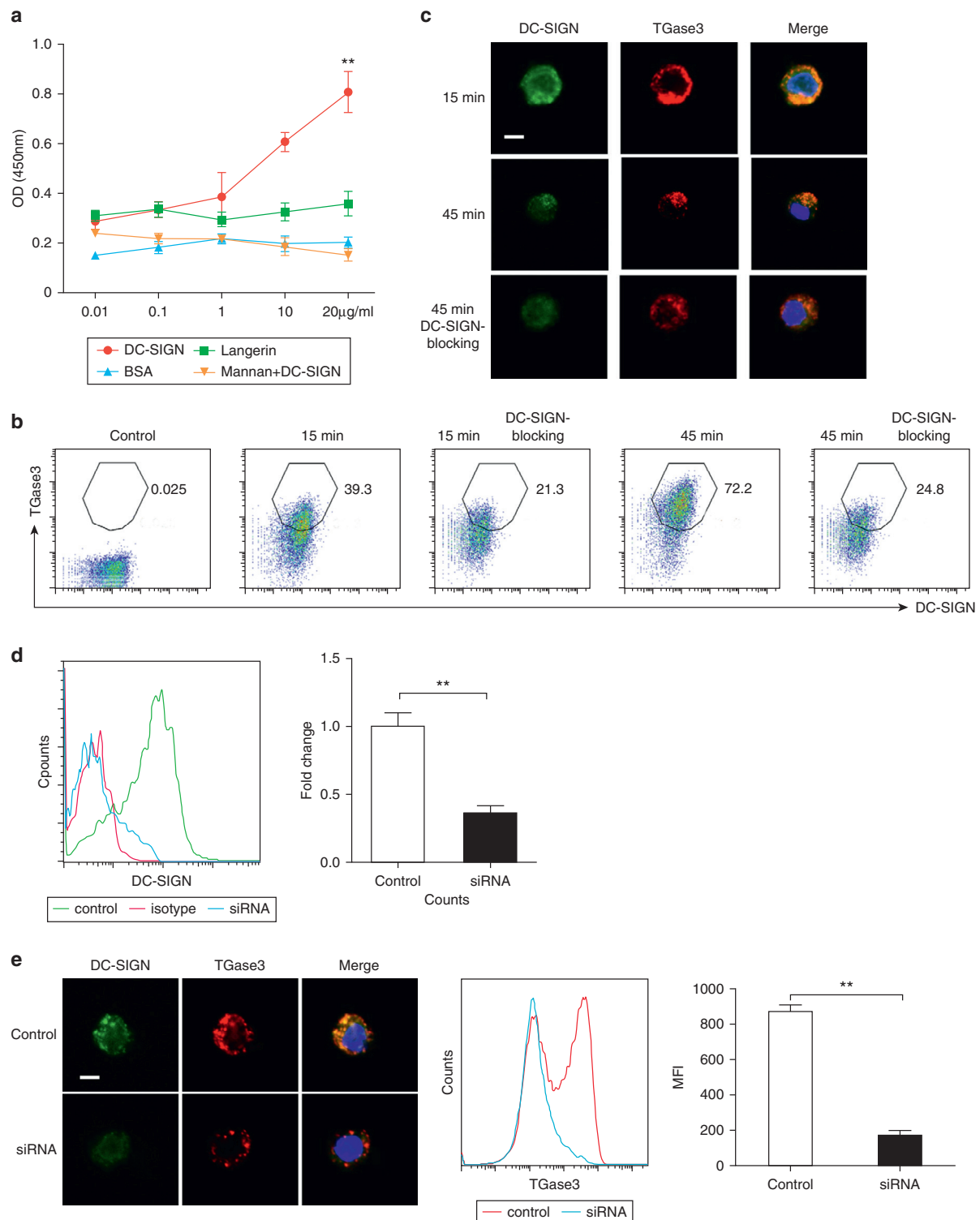


**Figure 3. Binding of TGase3 to DC-SIGN.** (a) Binding of TGase3 to soluble DC-SIGN-Fc or Langerin was revealed by ELISA. BSA was used as the negative control. The inhibition assay was performed by incubating DC-SIGN with Mannan for 1 hour prior to the addition to TGase3. The relative binding activity was expressed as the optical density (OD). (b) MoDCs were incubated with Alexa Fluor 647-conjugated TGase3 and FITC-conjugated anti-DC-SIGN antibody. Anti-DC-SIGN blocking antibody was used to confirm the specificity of TGase3 for DC-SIGN. (c) Immunofluorescence result showing the binding of TGase3 by MoDCs at different time points. Anti-DC-SIGN blocking antibody was used to confirm the specificity of TGase for DC-SIGN. Original magnification: 400X. Scale bar = 5 μm. Green: DC-SIGN; red: TGase3; blue: DAPI. (d) MoDCs were transfected with DC-SIGN-specific siRNA or control siRNA, and DC-SIGN expression was quantitatively analyzed by FCM and qRT-PCR. (e) MoDCs transfected with DC-SIGN-specific siRNA were incubated with Alexa Fluor 647-conjugated TGase3 and subjected to immunofluorescence and FCM analyses. Data shown are representative of at least three independent experiments, and statistical results are shown as the means ± standard deviation. \* $P < 0.05$ , \*\* $P < 0.01$ . BSA, bovine serum albumin; DC-SIGN, dendritic cell-specific; FCM, flow cytometry; MoDCs, monocytes-derived dendritic cells; OD, optical density; qRT-PCR, quantitative real-time RT-PCR; siRNA, small interfering RNA.

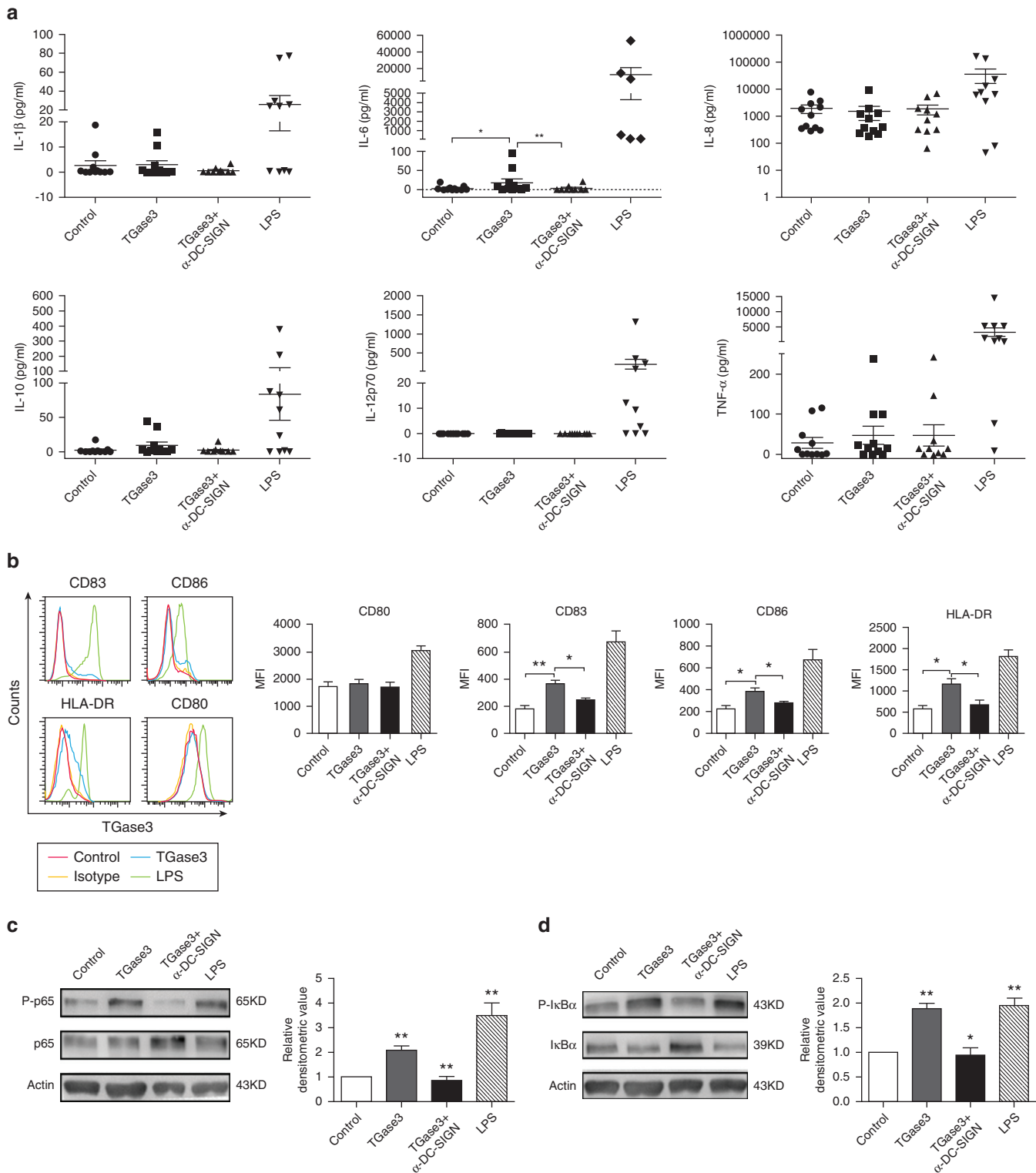


**Figure 4. TGase3 induced MoDCs activation via DC-SIGN.** MoDCs were incubated with TGase3 for 24 h, and anti-DC-SIGN blocking mAb was used to test the specificity of TGase3 to DC-SIGN. (a) Levels of IL-1 $\beta$ , IL-6, IL-8, IL-10, IL-12p70 and TNF- $\alpha$  in the culture supernatant were assessed by a cytometric beads array. (b) Surface expression of co-stimulatory molecules and HLA-DR were revealed by FCM. (c) Western blotting showing the expression of phospho-I $\kappa$ B $\alpha$  and phospho-p65 in the MoDCs. Data are representatives from at least three independent experiments and are expressed as the means  $\pm$  standard deviation. \* $P$  < 0.05, \*\* $P$  < 0.01. FCM, flow cytometry; MoDCs, monocytes-derived dendritic cells; TNF, tumour necrosis factor.

increase in the expression of IL-6. Nonspecific effects of anti-DC-SIGN Abs were excluded using isotype control Abs (Supplementary Figure 3). We then analyzed the expression of CD80, CD86, CD83 and HLA-DR at the same time point

in the cultured MoDCs by FCM, and the result showed that the expression of CD83, CD86 and HLA-DR was significantly increased in the TGase3-treated group (Figure 4b). We further explored the pro-inflammatory signalling pathway in MoDCs

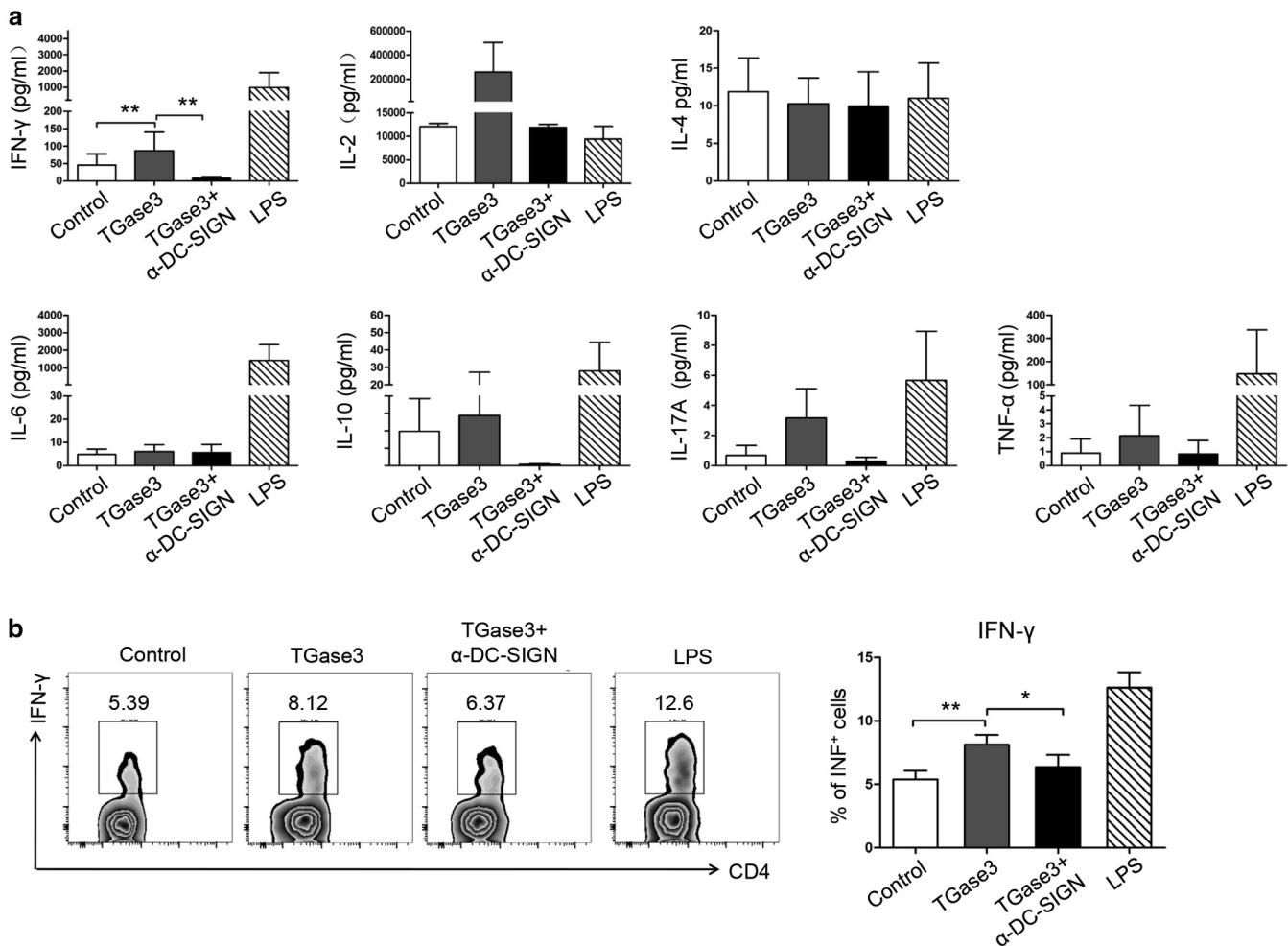


**Figure 5. T-cell differentiation induced by TGase3-stimulated MoDCs.** MoDCs from healthy individuals were first incubated with TGase3, then co-cultured with naïve autologous T cells for 2 weeks. Supernatant of the culture was harvested, and analyzed by cytometric beads array for IL-2, IL-4, IL-6, TNF- $\alpha$ , IL-10A, IFN- $\gamma$  and IL-17A (a), and by FCM for intracellular IFN- $\gamma$  (b). Data represent results from at least three independent experiments and are expressed as the means  $\pm$  standard deviation. \* $P$ <0.05, \*\* $P$ <0.01. MoDCs, monocytes-derived dendritic cells.

upon treatment with TGase3 and found that the expression of phospho-I $\kappa$ B $\alpha$  and phospho-p65 was significantly increased in the TGase3-treated group compared with those in the control group (Figure 4c and d), which indicated that the NF- $\kappa$ B signaling was activated by TGase3. Collectively, these data indicated that TGase3 might play a pro-inflammatory role in the pathogenesis of AD.

#### Th1 polarization induced by TGase 3-treated MoDCs

We then tested the subsequent effects of TGase3-activated MoDCs on T-cell differentiation. MoDCs stimulated with TGase3 were co-cultured with naïve autologous T cells for two weeks. Proliferation of T cells was determined by CFSE staining (Supplementary Figure 4), and T-cell differentiation was analyzed by the measurement of the cytokines, including IL-2, IL-4, IL-6, TNF- $\alpha$ , IL-10A, IFN- $\gamma$  and IL-17A. Consistent with the results from previous reports on autoantigens that induced Th1 response (Guttman-Yassky et al., 2011a), our study showed that the T cells produced increased levels of IFN- $\gamma$  in the presence of TGase3-treated MoDCs (Figure 5a). Intracellular staining for IFN- $\gamma$  was also assessed by FCM,

and the result was consistent with that of the cytokines (Figure 5b).

#### Inhibition of TGase3 attenuated skin inflammation in mouse model of AD

Lastly, the effects of TGase3 on AD were further assessed in the MC903-induced mouse model of AD using cysteamine, a TGases inhibitor, in C57BL/6 mice. Cysteamine (40 mg/kg) were intraperitoneally injected to the MC903-induced mouse model of AD once daily during the whole period of application with MC903 (Oh et al., 2013). The expression of TGase3 was significantly increased in the lesional skin of the AD model and was decreased upon treatment with cysteamine, which was similar to the level of the vehicle control (Figure 6a). Skin inflammation in the cysteamine-treated mice was significantly attenuated compared with that of the AD model, as shown by gross appearance (Figure 6b), ear thickness (Figure 6c), and histology (Figure 6d and e). The number of infiltrating Gr1 $^{+}$  cells in the regional draining lymph node of the mice in cysteamine group was significantly lower than that of the AD model (Figure 6f). The total IgE level in the mice after cysteamine treatment was also

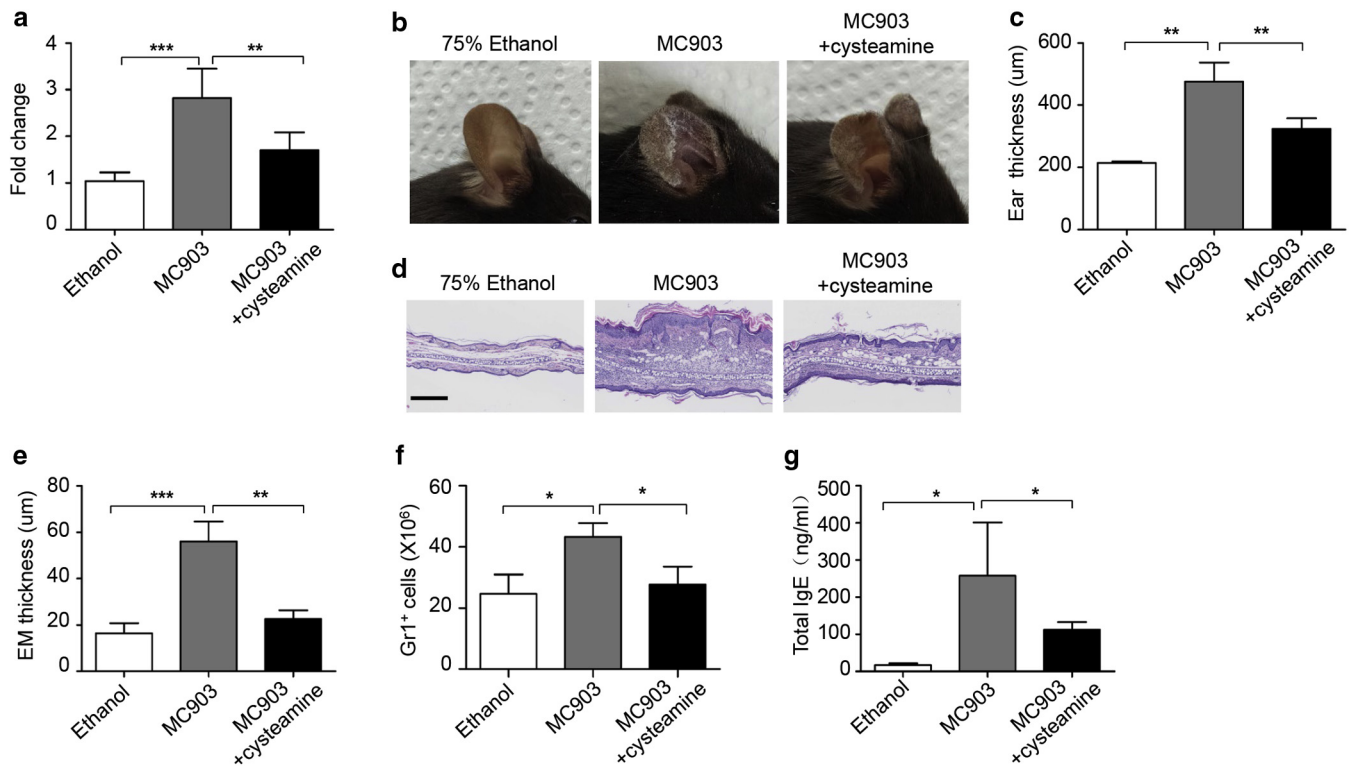


**Figure 6. Inhibition of TGase3 attenuated MC903-induced AD-like dermatitis of mice.** C57BL/6 mice were painted onto the ears with MC903 or ethanol control once daily for 12 days along with intraperitoneal injection with cysteamine. The expression level of TGase3 in the lesional skin was analyzed by qRT-PCR (a). Gross appearance of the ears (b), changes of the ear thickness (c), HE staining of the ear sections at day 14 (d), and epidermis (EM) thickness of the ear sections under high power magnification (e) were shown. Original magnification, 100X. Scale bar, 100  $\mu$ m. FCM showed the number of infiltrating Gr1<sup>+</sup> cells in the regional draining lymph node (f). Serum level of the total IgE was analyzed by ELISA (g). Data are representative of three independent experiments. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . AD, atopic dermatitis; FCM, flow cytometry; qRT-PCR, quantitative real-time RT-PCR.

lower than that of the AD model (Figure 6g). Similar effects of cysteamine were also shown when BALB/C mice were used (Supplementary Figure 5). These data indicated that skin inflammation in MC903-induced AD-like dermatitis was attenuated when the expression of TGase3 was inhibited, which was consistent with the pro-inflammatory effects of TGase3 in vitro.

## DISCUSSION

The term autoallergy denotes autoimmunity that is accompanied with an atopic disease, and increased levels of IgE against autoantigen is the hallmark (Hradetzky et al., 2015). AD patients with autoallergy are likely to be more severe and difficult to treat, and self-reactive IgE might be a possible contributing factor in the pathogenesis of AD (Tang et al., 2012). Autoantigens that are recognized by IgE have also been attracting extensive interest, and a number of autoallergens have been identified. Previous studies mainly focused on the identification and characterization of autoantigens recognized by IgE. However, how autoallergens are recognized by the immune system and what functions autoallergens play in the pathogenesis of AD remain largely unknown. In this study, TGase3 was uncovered as an autoallergen as revealed by the increased level of serum IgE against this protein. The expression of TGase3 in the lesional skin of AD patients was also increased. TGase3 activated the NF- $\kappa$ B signalling pathway in the MoDCs via the binding of

DC-SIGN, and Th1 polarization was subsequently induced. Our study revealed the autoreactivity to a barrier function-related protein of the skin in AD, which promoted skin inflammation in AD patients.

An increasing number of autoantigens that are recognized by IgE in AD have been identified, and these autoantigens can roughly be divided into three groups: autoantigens that are cross-reactive with exogenous allergens, that is, MnSOD; autoantigens that are identified by screening cDNA libraries using sera from AD patients; and autoantigens that are known from classical autoimmune diseases, such as bullous pemphigoid and Graves' disease (Hradetzky et al., 2015). It's interesting that TGase3 demonstrates features of all the three types of autoallergens: TGase3 shares a homologous sequence with Derf (Ichikawa et al., 1998); transglutaminase is included in the data of the cDNA library screened by the sera of AD (Zeller et al., 2009); TGase3 is recognized as an autoantigen in dermatitis herpetiformis. However, the exact mechanism for TGase3 to become an autoallergen is not yet clear. The features for an autoantigen to become autoallergen include molecular mimicry, auto-adjutant property, and protease activity (Hradetzky et al., 2015). The molecular mimicry does not seem to play a role in the production of anti-TGase3 IgE, as there is no correlation between the IgE against TGase3 and that against dust mite in AD patients. Upon binding to DC-SIGN, TGase3 induced the activation of the NF- $\kappa$ B signaling pathway and the production of IL-6 in

MoDCs, and the pro-inflammatory cytokine might be involved in the subsequent immune responses. The effect of TGase3 as auto-adjuvant is similar to that of Derp2, which binds the Toll-like receptor 4 and induces an allergic reaction (Trompette et al., 2009). TGase3 is also a functional enzyme that can catalyze the isopeptide bonds between glutamine and lysine residues in the substrate proteins in  $\text{Ca}^{2+}$ -dependent posttranslational modifying reaction (Tatsukawa et al., 2015), and the enzyme activity of TGase3 might also be involved in immune responses. Further experiments are needed to explore the mechanism by which TGase3 become an autoallergen in detail.

Our result is consistent with a previous study on the expression of TGase3, which shows that the expression of TGase3 is increased in the lesional skin of AD patients at the mRNA level (Liedén et al., 2012). It has been reported that the expression of TGase3 is increased upon stimulation with oxidative stress, mechanical trauma or UV radiation (Frezza et al., 2017). In this study, our result showed in an in vitro experiment that the increased expression of TGase3 was induced in keratinocytes under the Th2 inflammatory circumstance, in which IL-4, IL-1 $\beta$ , house dust mite, and house dust mite plus IL-4 were added mimicking the micro-environment of the AD skin. The detailed function for the increased expression of TGase3 in AD skin is not yet clear. As the principal role of TGase3 is to cross-link a number of structural proteins, including envoplakin, periplakin, loricrin, small proline-rich proteins, and filaggrin to form a cell envelope barrier structure essential for barrier function (Candi et al., 2005; Candi et al., 1999; Melino and Piacentini, 1998; Steinert et al., 1999), the increased expression of TGase3 in the skin lesions of AD might compensate for the disrupted barrier function. However, the cross-linking of self and (or) exogenous proteins by TGase3 may create novel antigens that may result in exacerbated immune responses compared with the proteins without cross-linking. A recent study reported that transglutaminase-induced cross-linking of tropomyosin, the major allergen in shellfish, changed the allergenicity of the allergen (Wang et al., 2019). The exact role of TGase3 as a cross-linking enzyme to modify the antigenicity of proteins in skin inflammation of AD is currently unclear and merits further exploration. When the expression of TGase3 was suppressed in the mouse model of AD by cysteamine, a transglutaminase inhibitor, skin inflammation was attenuated (Figure 6b-g), which further confirmed the pro-inflammatory role of TGase3 in AD. TGase3 may also participate in the pathogenesis of AD through anti-TGase3 IgE by cross-linking Fc $\epsilon$ RI that is expressed on mast cells, basophils, and Langerhans cells in the skin. The function of TGase3 in AD and dermatitis herpiformis may be different, as anti-TGase3 IgG but not IgA or IgM was increased in AD (Supplementary Figure 6). More studies are needed to elucidate the exact function of TGase3 in AD, and the inhibition of TGase3 expression and/or activation might be a therapeutic strategy for the treatment of AD.

The receptors mediating the recognition of autoallergen by the immune system remain poorly understood. Our results demonstrated that DC-SIGN, a CLR mainly expressed on DCs, mediated the binding and processing of TGase3 by MoDCs. It has been reported that DC-SIGN not only

recognizes N-glycan structures-expressing exogenous proteins but also several autoantigens (Clark et al., 2012). We previously found that DC-SIGN was able to bind several common allergens, including house dust mite allergen (Der p2) and egg white allergen (Gal d2) (Zhang et al., 2016). In this study, the result showed that there was a N-glycosylation site in TGase3, and TGase3 could bind DC-SIGN expressing on MoDCs. It has been shown that DC-SIGN-triggering activates the serine/threonine protein kinase RAF1, which induces the phosphorylation of the NF- $\kappa$ B subunit p65 at ser276 that functions as a binding site for the histone acetylase CBP; the acetylation of p65 induces enhanced and prolonged inflammatory factor transcription (Gringhuis et al., 2007). In accordance with previous studies, our results showed that the ligation of DC-SIGN by TGase3 induced increased expression of phospho-I $\kappa$ B $\alpha$  and phospho-p65 in the MoDCs, which was indicative of the activation of the NF- $\kappa$ B signaling pathway. DC-SIGN might be utilized as a therapeutic target for intervention on skin inflammation of AD.

It was suggested in previous studies investigating T cell responses to autoallergens that an intrinsic difference between exogenous allergens and autoallergens determines the direction of T-cell differentiation (Aichberger et al., 2005; Mittermann et al., 2008). Exogenous allergens preferentially induce Th2 responses, and autoallergens favor Th1 polarization (Guttman-Yassky et al., 2011a). In line with previous studies, our result showed that TGase3-activated MoDCs induced naïve T cells to differentiate to Th1 cells that were characterized by the increased expression of IFN- $\gamma$ . Keratinocytes of patients with AD exhibit increased IFN- $\gamma$ -induced apoptosis compared with keratinocytes from healthy subjects (Rebane et al., 2012), and the cell death-induced inflammation may in turn promote the infiltration of autoreactive T cells into the skin. Usually, the acute phase of skin lesions in AD is characterized by Th2 inflammation, whereas the chronic phase is characterized by mixed Th22, Th1, and Th17 inflammation (Guttman-Yassky et al., 2011b). The Th1 response induced by TGase3 indicated that the autoallergen may be mainly involved in the chronic phase of AD inflammation.

We propose a working model for the role of TGase3 in the pathogenesis of AD: in the microenvironment of AD skin, Th2 cytokines and/or allergen increase the expression of TGase3 in keratinocytes, and tissue damage and exogenous agents (e.g., scratching, superinfections) lead to the release of intracellular TGase3; TGase3 is presented to MoDCs via DC-SIGN, which activates the NF- $\kappa$ B signaling pathway and induces Th1 responses. IFN- $\gamma$  produced by T cells contributes to keratinocyte damage, perpetuating the chronicity of the disease. Further understanding of the detailed mechanism would result in the development of novel therapeutic strategies for the treatment of AD.

## MATERIALS AND METHODS

We provide a brief description of the methods below, with additional details in [Supplementary Material and Methods](#).

### Patient information

For the screening of specific IgE in sera, 46 AD patients and 59 healthy controls matched in sex and age were enrolled. The patients

fulfilled the diagnosis criteria of Hanifin and Rajka (Vakharia et al., 2018). The study was approved by the ethics committee of the Institute of Dermatology in the Chinese Academy of Medical Sciences, and written informed consent was obtained from the participants or their parents. Participants' information was listed in Supplementary Table 1. See also in the Supplementary Material and Methods.

### Capture ELISA for the detection of specific IgE

A Capture ELISA developed in-house was used to detect the serum-specific IgE as described previously (Sun et al., 2008). The procedure and the antibodies used are described in the Supplementary Material and Methods.

### Primary keratinocytes culture

Primary keratinocytes were prepared according to standard protocols, and the detailed procedure is described in the Supplementary Material and Methods.

### Quantitative real-time RT-PCR

The procedure and the primers are described in the Supplementary Material and Methods.

### Western blotting

The procedure and the antibodies used are described in the Supplementary Material and Methods.

### Immunofluorescence and confocal microscopy

The procedure and the antibodies used are described in the Supplementary Material and Methods.

### Solid phase binding assay

The procedure and the antibodies used are described in the Supplementary Material and Methods.

### N-linked glycans analysis

Detailed methods are described in the Supplementary Materials and Methods.

### MoDCs derivation and treatment

CD14<sup>+</sup> monocytes were separated from peripheral blood mononuclear cells using human CD14 MicroBeads (MACS, Miltenyi Biotec, San Diego, CA) and were cultured with 100 ng/ml GM-CSF and 20 ng/ml IL-4 (R&D Systems, Minneapolis, MN) to obtain DCs as described before (van den Heuvel et al., 1998). Detailed methods for the treatment of MoDCs are described in the Supplementary Materials and Methods.

### DC-T cell co-culture

The procedures are described in the Supplementary Material and Methods.

### AD mouse model and treatment

The animal experiments were performed in compliance with the guidelines outlined in the Guide for the Care and Use of Laboratory Animals of the Institute. MC903 (calcipotriol; Leo Pharma, Ballerup, Denmark)-induced AD model of mice were produced, and the mice were injected intraperitoneally with cysteamine (40 mg/kg/day, Sigma-Aldrich, St. Louis, MO) to inhibit TGase3 activity (Oh et al., 2013). The detailed procedure is described in the Supplementary Material and Methods.

### Statistical Analysis

The procedure is described in the Supplementary Material and Methods.

### Data availability statement

The authors declare that all the relevant data supporting the findings of this study are either available in this article and its supplementary files or from the corresponding author upon request.

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### CONFLICT OF INTEREST

The authors state no conflict of interest.

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### AUTHOR CONTRIBUTIONS

Conceptualization: XY, WL; Data Curation: HS, YL, XL, XY; Formal Analysis: HS, YL, XL, XY; Funding Acquisition: XY, WL, BW; Investigation: HS, YL, JS, XL, SL, BX, YZ, JL; Methodology: HS, YL, XL, XY; Project Administration: XY, WL, BW; Resources: XY, BW; Supervision: XY, BW; Validation: WL, YL; Visualization: WL, XY; Writing - Original Draft Preparation: HS, XY; Writing - Review and Editing: WL, and XY.

### SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at [www.jidonline.org](http://www.jidonline.org), and at <https://doi.org/10.1016/j.jid.2019.07.703>.

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## SUPPLEMENTARY MATERIALS AND METHODS

### Patients' information

For the screening of specific IgE against TGase3, tubulin and dust mite, 46 AD patients (aged 12-65 years, mean age 26.06 years) and 59 healthy controls (aged 12-64 years, mean age 29.07 years) matched in sex and age were enrolled. The patients fulfilled the diagnosis criteria of Hanifin and Rajka, with the Scoring Atopic Dermatitis Index (SCORAD) ranging from 15.1 to 88.5. For the analysis of TGase3 expression in the skin, biopsies of lesional skin were taken from six AD patients (mean SCORAD=46.37), and normal skin samples were obtained from nine sex- and age-matched healthy individuals undergoing plastic surgery. Peripheral blood was taken from 10 healthy individuals for the functional study of TGase3. All of the patients enrolled in our study had no autoimmune or systemic inflammatory diseases, and the skin lesions were not treated with any topical medications for at least 2 weeks before the biopsy. The study was approved by the ethics committee of the Institute of Dermatology in Chinese Academy of Medical Sciences, and written informed consent was obtained from the participants or their parents. Participants' information is listed in [Supplementary Table 1](#).

### Capture ELISA for detection of specific IgE

A Capture ELISA developed in-house was used to detect the serum-specific IgE as described previously. In brief, 2.5 µg/ml anti-human IgE was coated onto the microplates at 4 °C overnight, which was followed by blocking with 1% BSA/PBS for 2 h at room temperature. The plates were then incubated with sera samples for 2 hours, and biotin-labeled TGase3 (4 µg/ml) were added after washing and were then incubated with streptavidin-labeled horseradish peroxidase (HRP). The color reaction was developed with 3, 3', 5, 5'-Tetramethylbenzidine (eBioscience Inc, San Diego, CA) and terminated with 2 M H<sub>2</sub>SO<sub>4</sub>. Absorbance was measured at 450 nm by a plate-reader (Bio-Rad Laboratories, Inc, Hercules, CA, USA).

### Primary keratinocytes culture

The skin samples were incubated with 2.5 mg/ml Dispase (Gibco, Grand Island, NY) overnight at 4 °C to dissect the epidermis from the dermis, and the epidermis was then digested with 0.25% trypsin-EDTA (Gibco, Grand Island, NY) at 37 °C for 5 minutes. The cells were cultured in EpiLife medium (Invitrogen, Carlsbad, CA) which was supplemented with 1% human keratinocyte growth supplement, 100 IU/ml penicillin and 100 µg/ml streptomycin (Gibco, Grand Island, NY) in the presence of 60 µM of Ca<sup>2+</sup>. Primary human keratinocytes in culture were stimulated with IL-4 (20 ng/ml, R&D systems), Derp2 (10 µg/ml, Indoor Biotechnologies, Charlottesville, VA), IL-1β (20 ng/ml, R&D systems), or IL-4 plus Derp2 for 12 hours and then subjected to quantitative real-time RT-PCR (qRT-PCR) analysis.

### Solid phase binding assay

Microplates were coated with varying concentrations of TGase3 or tubulin (R&D Systems, Minneapolis, MN) and were blocked with 10% FBS. The plates were then incubated with 1 µg/ml recombinant soluble human DC-SIGN-Fc or Langerin (R&D Systems) followed by incubation with HRP-conjugated goat anti-human IgG Fc Abs (Millipore, Bedford, MA) or goat anti-human Langerin Abs (R&D

Systems). Then HRP-conjugated mouse anti-goat IgG Abs (Beyotime Biotechnology, Jiangsu, China) were added. Ten millimolar EDTA was added to test the blocking effect, and 1% BSA was used as a negative control. To confirm the binding specificity, inhibition assays were performed with a serial dilution of mannan (0-3.0 mg/ml, Sigma-Aldrich, St. Louis, MO), which was added and incubated for 1 hour before the antigens were added to the plates for binding assays. The relative binding activity was expressed as optical density (OD) for each test antigen after subtracting the values from the background.

### N-linked glycans analysis

The N-linked glycans analysis of recombinant human TGase3 (R&D Systems) was performed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) at the PTM BIO (Hangzhou, China). N-linked glycans were those released from resulting peptides/glycopeptides via digestion with peptide N-glycosidase F (PNGase F, New England Biolabs P0705L, Hitchin, United Kingdom). The tryptic peptides were separated with EASY-nLC 1000 UPLC system, and the peptides were subjected to NSI source followed by tandem mass spectrometry (MS/MS) in Q Exactive Plus (Thermo Scientific, Waltham, MA) coupled online to the UPLC. The detailed procedure are as follows:

*Gel electrophoresis and protein staining:* Recombinant human TGase 3 was run on 12% SDS-PAGE, and the gel was stained with a Coomassie Blue Staining Kit (Beyotime Biotechnology) according to the manufacturer's instructions.

*In-gel digestion:* The protein band was excised from the gel and cut into small pieces. For in-gel tryptic digestion, the gel pieces were destained in 50 mM NH<sub>4</sub>HCO<sub>3</sub> in 50% acetonitrile (v/v) until clear. The gel pieces were dehydrated with 100 µl of 100% acetonitrile for 5 minutes; the liquid was then removed, and the gel pieces were rehydrated in 10 mM dithiothreitol and incubated at 37 °C for 60 minutes. The gel pieces were again dehydrated in 100% acetonitrile, the liquid was removed, and the gel pieces were rehydrated with 55 mM iodoacetamide. The samples were incubated at room temperature in the dark for 45 minutes. The gel pieces were washed with 50 mM NH<sub>4</sub>HCO<sub>3</sub>, dehydrated with 100% acetonitrile, and then rehydrated with 10 ng/µl trypsin resuspended in 50 mM NH<sub>4</sub>HCO<sub>3</sub> on ice for 1 hour. Excess liquid was removed, and the gel pieces were digested with trypsin at 37 °C overnight. The peptides were extracted with 5% formic acid, followed by 100% acetonitrile. The free peptides were then dried to completion and resuspended in 0.1% formic acid. Two microliters of PNGaseF (New England Biolabs P0705L, UK) was added and incubated at 37 °C overnight. The samples were dried further under vacuum overnight before use.

*LC-MS/MS analysis:* The tryptic peptides were dissolved in solvent A (2% acetonitrile, 0.1% formic acid) and directly loaded onto a home-made reversed-phase analytical column (15 cm length, 75 µm i.d.). The gradient was comprised of an increase from 6% to 23% solvent B (90% acetonitrile, 0.1% formic acid) over 16 minutes, 23% to 35% in 8 minutes and increasing to 80% in 3 minutes then holding at 80% for the last 3 minutes, all at a constant flow rate of 400 nL/min on an EASY-nLC 1000 UPLC system.

The peptides were subjected to NSI source followed by tandem mass spectrometry (MS/MS) in Q Exactive Plus (Thermo Scientific) coupled online to the UPLC. The electrospray voltage applied was 2.0 kV. The *m/z* scan range was 350 to 1800 Da for the full scan, and intact peptides were detected in the Orbitrap at a resolution of 70,000. Peptides were then selected for MS/MS using an NCE setting as 28, and the fragments were detected in the Orbitrap at a resolution of 17,500. A data-dependent procedure that alternated between one MS scan was followed by 20 MS/MS scans with 15.0 s dynamic exclusion. Automatic gain control (AGC) was set at 5E4.

**Data processing:** The resulting MS/MS data were processed using Proteome Discoverer 1.3 (Thermo Fisher Scientific, Waltham, MA). Tandem mass spectra were searched against the TGase3 database. Trypsin/P was specified as the cleavage enzyme allowing up to 2 missing cleavages. The mass error was set to 10 ppm for the precursor ions and 0.02 Da for the fragment ions. Carbamidomethyl on Cys were specified as a fixed modification, and oxidation on Met and TGase 3 modifications were specified as variable modifications. Peptide confidence was set at high, and the peptide ion score was set > 20.

#### Quantitative real-time RT-PCR

RNA extractions of cells or tissue samples were performed using TRIzol Reagent (Invitrogen, Life Technologies, CA) according to the manufacturer's instructions. Five hundred nanograms of RNA fraction was reversely transcribed to cDNA using PrimeScript RT Master Mix (TaKaRa, Dalian, China). The qRT-PCR assay was performed according to the manufacturer's instructions (7300 Sequence Detection System; Applied Biosystems) with primers and SYBR Green Master Mix (High ROX Premixed, Vazyme, Jiangsu, China). Threshold cycle (CT) values were used to calculate the fold change using the  $2^{-\Delta\Delta CT}$  method. The relative mRNA expression was normalized to the  $\beta$ -actin gene. The primer sequences are listed in [Supplementary Table S2](#).

#### Western blotting

Cells or tissues were lysed in cell lysis solution (DSL; Webster, Beyotime, Shanghai, China). The total protein concentration in the lysates was measured using the BCA Protein Assay kit (Pierce, Rockford, IL). Equal amounts of protein (50  $\mu$ g) were separated by 12% SDS-PAGE (Bio-Rad Laboratories, Inc) and then transferred to a PVDF membrane (Millipore). The membrane was incubated with primary antibodies overnight at 4 °C after blocking with 5% BSA. The following primary antibodies were used: sheep anti-TGase3 polyclonal Ab (4  $\mu$ g/ml, R&D systems), rabbit anti-phospho-IkB $\alpha$  mAb, mouse anti-IkB $\alpha$  mAb, rabbit anti-phospho-p65mAb, rabbit anti-p65mAb, and mouse anti- $\beta$ -actin mAb (1:1000, Cell Signaling Technology, Beverly, MA). After washing, the membrane was incubated with HRP-conjugated secondary antibodies (goat anti-rabbit IgG, 1:2000; horse anti-mouse IgG, 1:2000, Cell Signaling Technology) for 1 hour at room temperature. A Page Ruler Plus Prestained Protein Ladder (Fermentas, Hanover, USA) was used to confirm the protein electrophoresis and transfer. The labeled proteins were detected using the ECL western blotting detection system (Bio-Rad Laboratories, Inc). Image J

software (NIH, Bethesda, MD) was used to quantify the relative expression level of proteins by determining a ratio between the amount of the proteins and  $\beta$ -actin.

#### Immunofluorescence and confocal microscopy

Frozen sections were prepared from OCT-embedded skin tissues, blocked with 10% FBS, and incubated with goat anti-human TGase3 (Abcam, Cambridge, UK) overnight at 4 °C. After washing, the sections were incubated with Alexa Fluor 647-conjugated donkey anti-goat IgG (Invitrogen, Thermo Fisher Scientific) for 1 h at 37 °C in the dark. The sections were stained with DAPI (Roche Diagnostics, Chicago) and were imaged by confocal microscopy (Olympus FV1000, Olympus, Webster, TX).

#### MoDCs derivation and treatment

CD14<sup>+</sup> monocytes were separated from peripheral blood mononuclear cells using human CD14 MicroBeads (MACS, Miltenyi Biotec) and were cultured with 100 ng/ml GM-CSF and 20 ng/ml IL-4 (R&D Systems) to obtain DCs as described before. On day 6, the MoDCs were collected and incubated with Alexa Fluor 647-labeled TGase3 (10  $\mu$ g/ml) and rabbit anti-human DC-SIGN (Abcam, Cambridge, United Kingdom) for 15 minutes or 45 minutes at 37 °C, and analyzed by flow cytometry FCM(BD FACS Verse) and a confocal laser scanning microscope system (FluoViewTM FV 1000, Olympus corporation). For anti-DC-SIGN-blocking experiments, the MoDCs were pre-incubated with anti-DC-SIGN blocking antibody (5  $\mu$ g/ml) (MAB161, R&D System) for 30 minutes at 37 °C before incubation with TGase3. For an RNA interference assay, the MoDCs were transfected with DC-SIGN-specific small interfering RNA (siRNA) (50 nm) using Lipofectamine RNAiMax (Invitrogen) according to manufacturer's instructions. The oligonucleotides used are listed in [Supplementary Table 3](#). The efficiency of the gene knock-down was determined by qRT-PCR and FCM.

The MoDCs were cultured with the medium containing TGase3 (10  $\mu$ g/ml) for 24 hours. The level of IL-1 $\beta$ , IL-6, IL-8, IL-10, IL-12p70 and TNF- $\alpha$  in the supernatant was measured by a Cytometric Bead Array (CBA) Human Inflammatory cytokine kit according to the manufacturer's instructions (Catalog No.560484, BD Biosciences). The expression of CD80, CD86, HLA-DR, and CD83 was analyzed by FCM. Anti-DC-SIGN blocking antibody (5  $\mu$ g/ml) (MAB161, R&D System) was used to assess the specificity of DC-SIGN.

#### DC-T cell co-culture

After stimulation with TGase3 (10  $\mu$ g/ml, Catalog No. 4604-TG, R&D System) for 24 hours,  $3 \times 10^5$  MoDCs were cultured with  $1.5 \times 10^6$  purified CD4<sup>+</sup>CD45RA<sup>+</sup> T cells that were sorted by a naïve T cell selection kit (MACS, Miltenyi Biotec) from peripheral blood mononuclear cells of the same donor. Recombinant human-IL-2 (20 ng/ml, R&D System) was added to the culture every three days from day 5 to day 13. The proliferation of T cells was analyzed by a CellTrace™ CFSE Cell Proliferation Kit (Thermo Fisher). The levels of IL-4, IL-6, TNF- $\alpha$ , IL-10A, IFN- $\gamma$  and IL-17A in the supernatant on day 14 were measured by a CBA Human Inflammatory cytokine kit (Catalog No.551811, BD Biosciences). The cultured cells were collected for intracellular IFN- $\gamma$  (Catalog No.11-7319-81, eBioscience) analysis by flow cytometry.

**AD mouse model and treatment**

Wide type C57BL/6 mice and BALB/C mice were purchased from the Vital River Company (Import commission agent of Charles River Laboratories, Beijing). All the mice were 8-12 weeks old and kept on a 12 hour light/dark cycle under specific pathogen-free conditions. The animal experiments were performed in compliance with the guidelines outlined in the Guide for the Care and Use of Laboratory Animals of the Institute. Two nanomoles MC903 (calcipotriol; Leo Pharma, Ballerup, Denmark) dissolved in ethanol were topically applied onto mice ears consecutively for 12 days. The mice were injected intraperitoneally with cysteamine (40 mg/kg/day, Sigma-Aldrich) to inhibit TGase3 activity. Full-thickness ears were measured by a micrometer. The total IgE was measured by ELISA (Catalog No. 555248, BD,

Pharmingen, San Diego, CA). The skin tissues were fixed in formalin for histopathology analysis. The local lymph nodes were digested to acquire single-cell suspensions for further FCM analysis.

**Statistical Analysis**

The Student's t-test was used to compare the difference between paired and unpaired observations, and multi-group data were statistically analyzed using an analysis of variance test. Correlation between the parameter was performed by a Pearson correlation analysis. Quantitative data were presented as mean  $\pm$  standard deviation. A *P* value of  $< 0.05$  was considered statistically significant. SPSS 20.0 (Inc., Chicago, IL) and Graph Pad Prism 4.0 (San Diego, CA) were used for all the statistical analyses.

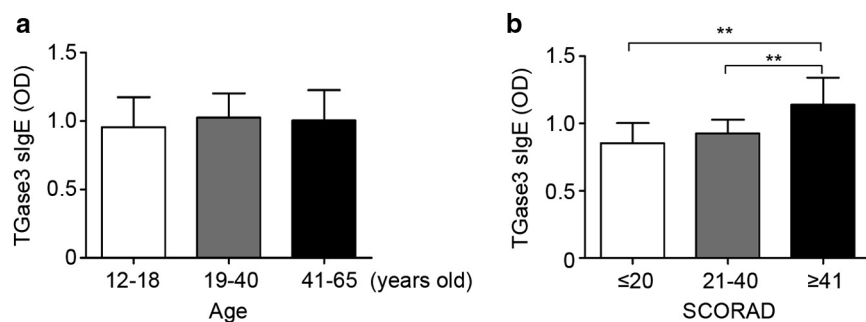


**Figure S1. TGase3-specific IgE of AD patients stratified by age and disease severity.** (a), AD patients were divided into three age groups, and the levels of TGase3-specific IgE were compared. 12-18-years old,  $n = 13$ ; 19-40 years old,  $n = 27$ ; 41-65 years old,  $n = 6$ . (b), AD patients were stratified into three degrees of severity, and the levels of TGase3-specific IgE were compared. SCORAD  $\leq 20$ ,  $n = 6$ ; SCORAD 21-40,  $n = 19$ ; SCORAD  $\geq 41$ ,  $n = 21$ . Data shown are mean  $\pm$  standard deviation. \* $P < 0.05$ . AD, atopic dermatitis; SCORAD, Scoring Atopic Dermatitis Index.

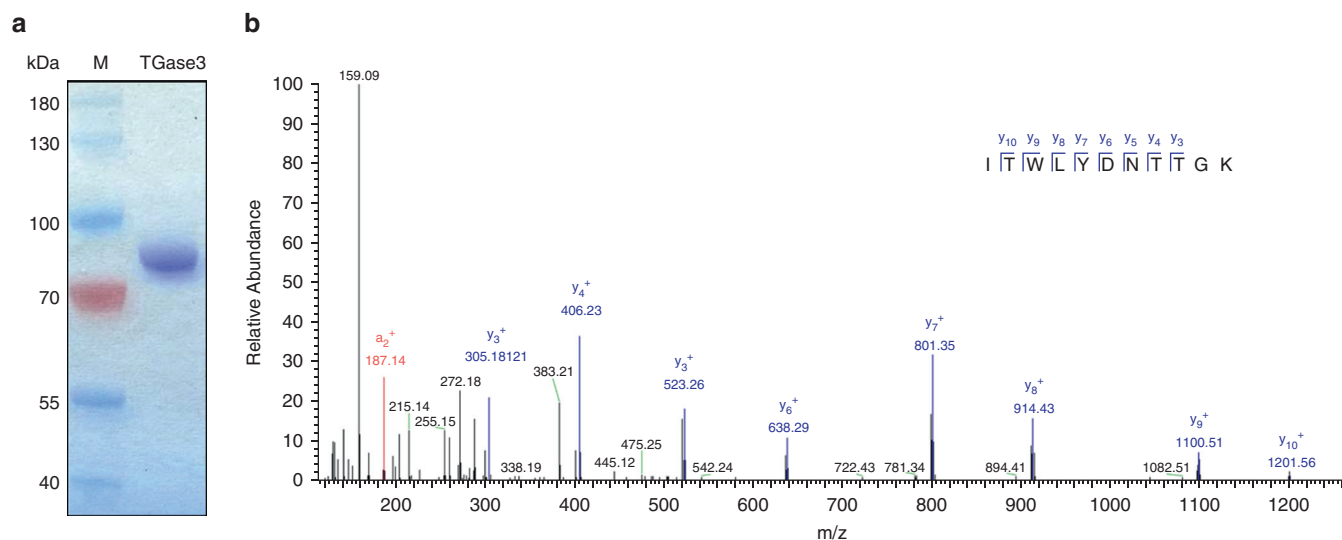


**Figure S2. Mass spectrum analysis of TGase 3.** (a), Purified TGase 3 was run on SDS-PAGE, stained with Coomassie blue. M, marker. (b) Mass spectrum analysis of the digested TGase 3 revealing a peak of  $m/z$  657.83 $^{2+}$ , and further inspection showing one N-glycosylation site at position 404 (ITWLYDNTTGK, N7 Deamidated:  $^{18}O$ ) with a modification of 2.99 Da.

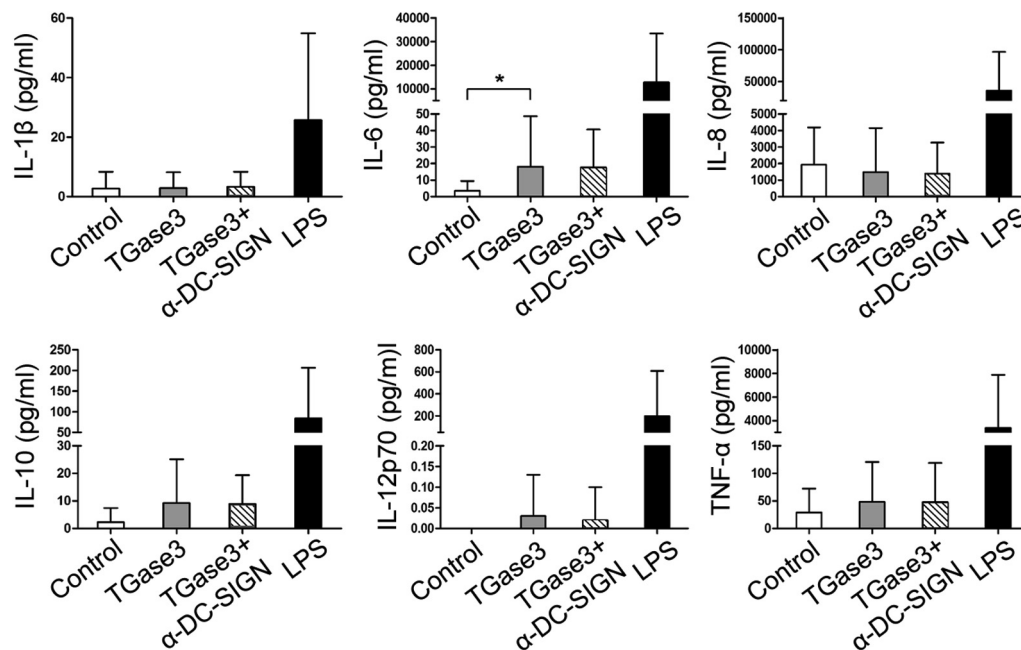
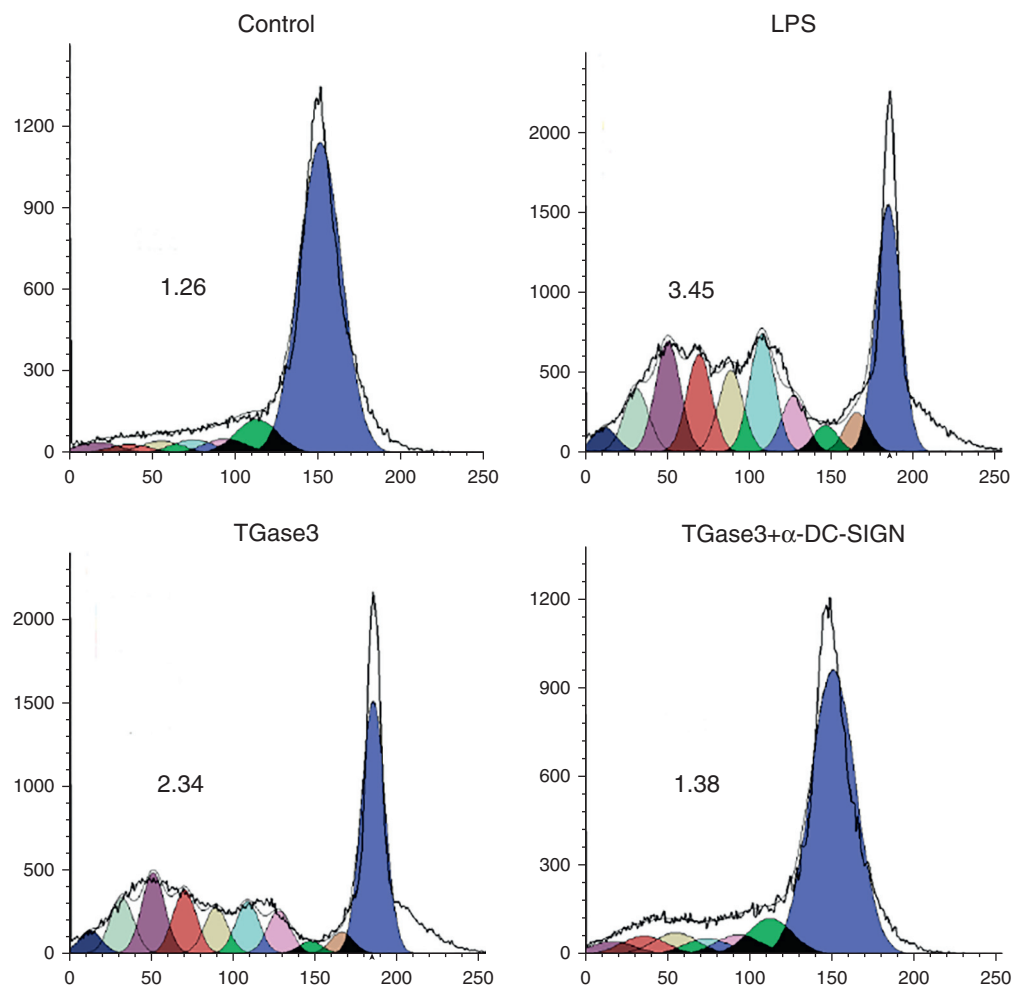


**Figure S3. Production of cytokines by MoDCs stimulated with TGase3.** The MoDCs were incubated with TGase3, TGase3 plus the isotype control for anti-DC-SIGN blocking antibody, and LPS for 24 hours, and the supernatant was subjected for the analysis of IL-1 $\beta$ , IL-6, IL-8, IL-10, IL-12p70 and TNF- $\alpha$  by CBA. Data represent the results from three independent experiments and are expressed as the mean  $\pm$  standard deviation. \* $P < 0.05$ . CBA, Cytometric Bead Array.

**Figure S4. Proliferation of T cells in co-culture with MoDCs.** Naïve T cells were stained with CFSE, and co-cultured with TGase3-stimulated MoDCs for 5 days. The cells were then labeled with anti-CD4 antibody and subjected to FCM. CFSE, Carboxyfluorescein succinimidyl ester; FCM, flow cytometry; MoDCs, monocytes-derived dendritic cells.



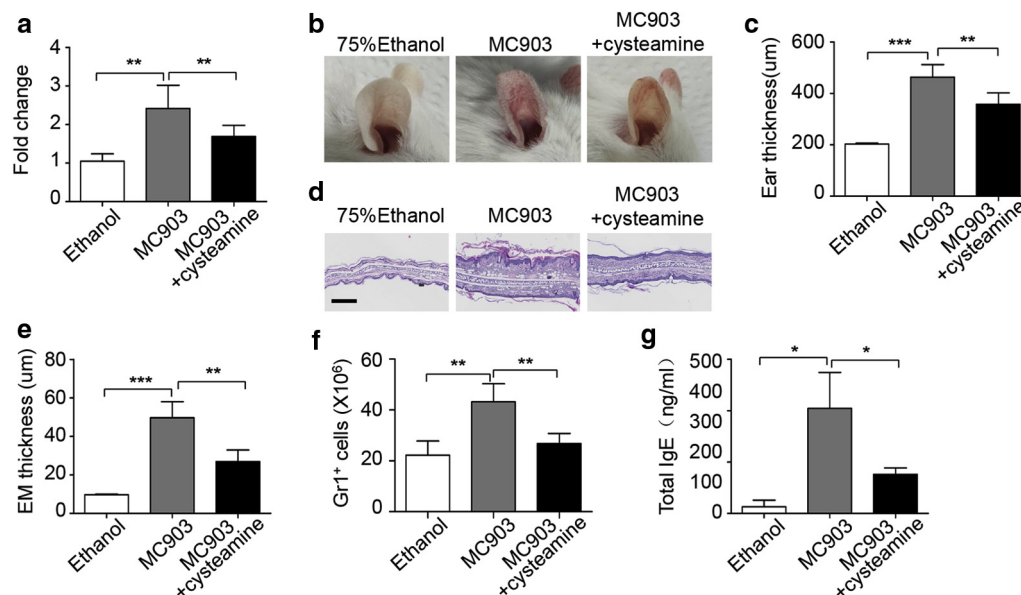


**Figure S5. Inhibition of TGase3 attenuated MC903-induced AD-like dermatitis in Balb/c mice.** Balb/c mice were painted onto the ears with MC903 or ethanol control once daily for 12 days, along with intraperitoneal injection with cysteamine. The expression level of TGase3 in the lesional skin was analyzed by qRT-PCR (a). Gross appearance of the ears (b), changes of the ear thickness (c), HE staining of the ear sections at day 14 (d), and epidermis (EM) thickness of the ear sections under high power magnification (e) were shown. Original magnification, 100X. Scale bar, 100 μm. FCM showed the number of infiltrating Gr1<sup>+</sup> cells in the regional draining lymph node (f). Serum level of total IgE was analyzed by ELISA (g). Data are representative of three independent experiments. \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001. AD, atopic dermatitis; FCM, flow cytometry; qRT-PCR, quantitative real-time RT-PCR.

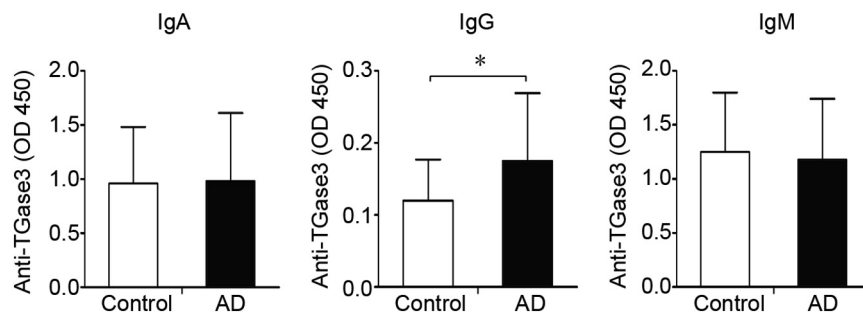


**Figure S6. Isotypes of anti-TGase3 antibody in AD patients.** Mean OD value of the anti-TGase3 antibodies in the sera of AD patients and healthy controls. AD, n = 46; healthy controls, n = 59. \**P* < 0.05. AD, atopic dermatitis; OD, optical density.

**Table S1. Information of the AD Patients and Healthy Controls****AD patients**

| Number | Gender (M=1, F=2) | Age | SCORAD | TGase3slgE (OD) |
|--------|-------------------|-----|--------|-----------------|
| 1      | 2                 | 12  | 23     | 0.853377        |
| 2      | 1                 | 12  | 15.1   | 0.719489        |
| 3      | 1                 | 12  | 67.5   | 1.08651251      |
| 4      | 1                 | 13  | 81     | 1.182294        |
| 5      | 2                 | 13  | 19.8   | 0.694115384     |
| 6      | 2                 | 14  | 39.2   | 0.84272         |
| 7      | 2                 | 14  | 46.1   | 1.002407        |
| 8      | 2                 | 14  | 52.5   | 1.115610789     |
| 9      | 2                 | 15  | 45.8   | 1.370594348     |
| 10     | 1                 | 15  | 26.8   | 0.811725        |
| 11     | 1                 | 16  | 50.8   | 1.252452845     |
| 12     | 1                 | 17  | 29     | 0.719560861     |
| 13     | 1                 | 18  | 58     | 0.860682        |
| 14     | 2                 | 19  | 41.8   | 0.843673        |
| 15     | 2                 | 19  | 53     | 1.014096        |
| 16     | 2                 | 19  | 63     | 1.435423        |
| 17     | 1                 | 20  | 32.4   | 0.957874447     |
| 18     | 2                 | 21  | 31.7   | 0.935539        |
| 19     | 1                 | 21  | 32     | 0.939271882     |
| 20     | 1                 | 21  | 37.4   | 0.879048883     |
| 21     | 2                 | 22  | 17.7   | 0.832534584     |
| 22     | 2                 | 22  | 43.6   | 1.19493842      |
| 23     | 2                 | 23  | 31     | 1.137478858     |
| 24     | 2                 | 23  | 88.5   | 1.308798        |
| 25     | 1                 | 24  | 42     | 0.993125177     |
| 26     | 1                 | 24  | 19.8   | 0.863324188     |
| 27     | 2                 | 24  | 26.2   | 0.925283767     |
| 28     | 2                 | 26  | 32.2   | 1.09703504      |
| 29     | 2                 | 27  | 51.5   | 1.020517607     |
| 30     | 2                 | 27  | 38.9   | 0.99523179      |
| 31     | 2                 | 27  | 34.2   | 0.949785        |
| 32     | 2                 | 27  | 44     | 1.044661793     |
| 33     | 1                 | 27  | 35.2   | 0.955347        |
| 34     | 2                 | 29  | 49.4   | 1.146108        |
| 35     | 2                 | 29  | 69     | 1.614593        |
| 36     | 2                 | 31  | 34     | 0.956292        |
| 37     | 2                 | 32  | 20.9   | 0.95127218      |
| 38     | 2                 | 36  | 18.2   | 1.104360905     |
| 39     | 1                 | 37  | 20     | 0.905788        |
| 40     | 1                 | 38  | 49.5   | 1.161943        |
| 41     | 2                 | 42  | 43     | 0.8731          |
| 42     | 2                 | 44  | 38.8   | 1.033538        |
| 43     | 1                 | 47  | 71     | 1.084901727     |
| 44     | 1                 | 58  | 31.9   | 0.776211135     |
| 45     | 2                 | 63  | 63     | 1.392304434     |
| 46     | 2                 | 65  | 33.4   | 0.86497643      |

**Healthy controls**

| Number | Gender (M=1, F=2) | Age | TGase3slgE (OD) |
|--------|-------------------|-----|-----------------|
| 1      | 1                 | 12  | 0.68834603      |
| 2      | 1                 | 12  | 0.57388535      |
| 3      | 2                 | 12  | 0.50453773      |
| 4      | 2                 | 13  | 0.5859754       |
| 5      | 2                 | 13  | 0.92370837      |

(continued)

**Table S1. Continued****Healthy controls**

| Number | Gender (M=1, F=2) | Age | TGase3slgE (OD) |
|--------|-------------------|-----|-----------------|
| 6      | 1                 | 13  | 0.83057719      |
| 7      | 2                 | 14  | 0.76317366      |
| 8      | 1                 | 14  | 0.89675432      |
| 9      | 2                 | 14  | 0.80009418      |
| 10     | 2                 | 14  | 0.63340977      |
| 11     | 2                 | 15  | 0.93338827      |
| 12     | 1                 | 15  | 0.48971497      |
| 13     | 1                 | 16  | 0.36815786      |
| 14     | 1                 | 17  | 0.84245302      |
| 15     | 2                 | 17  | 0.89629103      |
| 16     | 1                 | 18  | 0.67623443      |
| 17     | 2                 | 18  | 0.71464562      |
| 18     | 2                 | 18  | 0.7309824       |
| 19     | 2                 | 19  | 0.83402504      |
| 20     | 1                 | 19  | 0.91168821      |
| 21     | 2                 | 19  | 0.75662559      |
| 22     | 2                 | 20  | 0.71400866      |
| 23     | 1                 | 21  | 0.7884412       |
| 24     | 2                 | 21  | 0.69741961      |
| 25     | 2                 | 22  | 0.75500623      |
| 26     | 1                 | 22  | 0.65810774      |
| 27     | 2                 | 22  | 0.74528411      |
| 28     | 2                 | 24  | 0.88719734      |
| 29     | 2                 | 24  | 0.75371498      |
| 30     | 2                 | 25  | 0.62847841      |
| 31     | 1                 | 25  | 0.38232962      |
| 32     | 2                 | 26  | 0.84530194      |
| 33     | 1                 | 26  | 0.80738065      |
| 34     | 2                 | 27  | 0.84969581      |
| 35     | 2                 | 28  | 0.9013328       |
| 36     | 2                 | 28  | 1.03196749      |
| 37     | 2                 | 28  | 0.99148457      |
| 38     | 2                 | 30  | 1.01988825      |
| 39     | 2                 | 32  | 0.61533554      |
| 40     | 1                 | 34  | 0.67042364      |
| 41     | 1                 | 35  | 0.54118586      |
| 42     | 1                 | 38  | 0.49078215      |
| 43     | 2                 | 40  | 0.61945968      |
| 44     | 1                 | 40  | 0.61386222      |
| 45     | 1                 | 42  | 0.70303798      |
| 46     | 2                 | 42  | 0.64336133      |
| 47     | 2                 | 43  | 0.68530543      |
| 48     | 1                 | 45  | 0.77212849      |
| 49     | 2                 | 46  | 0.70776116      |
| 50     | 1                 | 48  | 0.64970511      |
| 51     | 1                 | 48  | 0.54115378      |
| 52     | 2                 | 49  | 0.71606701      |
| 53     | 1                 | 50  | 0.75328308      |
| 54     | 1                 | 52  | 0.63482614      |
| 55     | 2                 | 52  | 0.578257        |
| 56     | 2                 | 54  | 0.50403394      |
| 57     | 2                 | 60  | 0.52084694      |
| 58     | 2                 | 60  | 0.43970405      |
| 59     | 2                 | 64  | 0.55527289      |

Abbreviations: AD, atopic dermatitis; OD, optical density; SCORAD, Scoring Atopic Dermatitis Index.

**Table S2. Sequences of qRT-PCR Primers**

| Gene name      | Primer sequences                                         |
|----------------|----------------------------------------------------------|
| TGase 3        | F: TGGCAATACTCTGACTATCAGCA<br>R: GTATGAACGTCCCAAGTTTCACA |
| DC-SIGN        | F:TCAAGCAGTATTGGAACAGAGGA<br>R: CAGGAGGCTGCGGACTTTTT     |
| $\beta$ -actin | F: TCTGGCACCACACCTTCTA<br>R: AGGCATACAGGGACAGCAC         |

Abbreviations: DC-SIGN, dendritic cell-specific; qRT-PCR, quantitative real-time RT-PCR.

**Table S3. Sequences for siRNA**

| Gene              | Sequence                                                                                    |
|-------------------|---------------------------------------------------------------------------------------------|
| A:CD209 HSS121249 | sense-1:GGC AAU GGC UGG AAC GAC GAC AAA U<br>antisense-1:AAU UGU CGU CGU UCC AGC CAU UGC C  |
| B:CD209 HSS121250 | sense-2:UCC AGG GAU GAA CAG UUU CUU U<br>antisense-2:AAA GAA ACU GUU CUU CAU CCC UGG A      |
| C:CD209 HSS121251 | Sense-3: CUU GGA UGG GAC UUU CAG AUC UAA A<br>Antisense-3:UUU AGA UCU GAA AGU CCC AUC CAG G |

Abbreviation: siRNA, small interfering RNA.