Mast Cells Initiate Type 2 Inflammation through Tryptase Released by MRGPRX2/MRGPRB2 Activation in Atopic Dermatitis

Tao Jia[1,3], Delu Che[1,2,3], Yi Zheng[1], Huan Zhang[1], Yaxiang Li[1], Tong Zhou[1], Bin Peng[1], Xueshan Du[1], Longfei Zhu[1], Jingang An[1] and Songmei Geng[1]

Atopic dermatitis (AD) is a common chronic inflammatory skin disease characterized by T helper 2 inflammation as the core pathogenic mechanism. MRGPRX2 plays a key role in nonhistamine allergies and neuroimmune mechanisms in chronic inflammatory dermatitis. However, the role of MRGPRX2 in AD and the development of type 2 inflammation is not yet clear. This study aimed to define the role of MRGPRX2 in type 2 inflammation development and cytokine release in AD by determining its levels in patients with AD and healthy controls. Furthermore, *MrgprB2*-conditional knockout (*MrgprB2*$^{-/-}$) and wild-type mice were used to construct an MC903-induced AD mouse model to observe skin inflammation and cytokine release. Tryptase and its antagonist were applied separately to *MrgprB2*$^{-/-}$ mice with AD and wild-type mice with AD to confirm the role of the MRGPRB2–tryptase axis in the development of type 2 inflammation in AD. We found that AD severity and type 2 cytokine levels were not associated with IgE levels but were associated with MRGPRX2/MRGPRB2 expression. *MrgprB2*$^{-/-}$ mice with AD showed milder phenotypes and inflammatory infiltration in the skin than wild-type mice with AD. Tryptase released by MRGPRX2/MRGPRB2 activation is involved in the release of type 2 cytokines, which contributes to inflammatory development in AD.

Journal of Investigative Dermatology (2024) **144,** 53–62; doi:10.1016/j.jid.2023.06.201

INTRODUCTION

Atopic dermatitis (AD) is a chronic recurrent inflammatory skin disease that affects 15–20% of children and up to 10% of adults (Arents et al., 2022). The pathogenesis of AD is often accompanied by allergic asthma, allergic rhinoconjunctivitis, and other immediate hypersensitivities (Ständer, 2021). Furthermore, AD pathogenesis involves interactions between genetic and environmental factors, immune dysregulation, microbial imbalance, skin barrier dysfunction, and environmental triggers of skin inflammation. The T helper 2–dominated response is thought to be the inflammatory core of AD and associated type 2 cytokines include IL-4, IL-13, and thymic stromal lymphopoietin (TSLP) (Langan et al., 2020).

Mast cells (MCs) play a key role in allergic reactions and are considered the first line of defense against pathogens. Activated MCs can secrete mediators and recruit effector cells, which are crucial for host defense and immune function (Voss et al., 2021). Notably, MCs have been shown to increase AD (Imayama et al., 1995) and participate in AD pathogenesis through activation by allergens and IgE/FcεRI crossing (Metzger, 1992). However, antihistamine intake or inhibition of IgE-dependent pathways of MC activation does not efficiently relieve skin inflammation and itching in AD (Voss et al., 2021), suggesting that non-IgE–dependent activation of MCs plays a vital role in AD.

MRGPRX2 is a multiligand receptor expressed on skin MCs that responds to many endogenous and exogenous stimulants. In mice and rats, the MRGPRX2 ortholog is called MRGPRB2 (Kühn et al., 2021). MRGPRB2 has been shown to contribute to neuroimmune interactions in an AD mouse model (Thapaliya et al., 2021). MRGPRX2/MRGPRB2 activation can trigger MCs to release various enzymes, including tryptase (Meixiong et al., 2019). Tryptase, the most abundant mediator stored in MC granules, is a marker of MC activation and is related to allergic and anaphylactoid reactions (Payne and Kam, 2004). A recent study showed that tryptase can induce TSLP production by stimulating keratocytes in vitro (Redhu et al., 2022).

Determination of whether MRGPRX2/MRGPRB2 in MCs is involved in type 2 inflammation in AD and intractable pruritus through tryptase release is important for a better understanding of AD pathogenesis. The topical application of MC903 (calcipotriol) to mouse skin recapitulates the features of human AD (Moosbrugger-Martinz et al., 2017; Svanberg et al., 2020). This study used *MrgprB2*$^{-/-}$ mice to establish an AD mouse model to further investigate the role of MRGPRX2/MRGPRB2 in type 2 inflammation in AD.

RESULTS

AD severity and type 2 cytokine release were not strongly associated with IgE level

To investigate the biomarkers of AD severity in patients, the Visual Analog Scale and Eczema Area and Severity Index

[1]Department of Dermatology, Northwest Hospital, The Second Hospital Affiliated to Xi'an Jiaotong University, Xi'an, China; and [2]Center for Dermatology Disease, Precision Medical Institute, Xi'an, China

[3]These authors contributed equally to this work.

Correspondence: Songmei Geng, Department of Dermatology, The Second Hospital Affiliated to Xi'an Jiaotong University, 157 Xiwu Road, Xi'an 710004, China. E-mail: gengsongmei73@163.com

Abbreviation: AD, atopic dermatitis; ADI, Atopic Dermatitis Index; MC, mast cell; TSLP, thymic stromal lymphopoietin; WT, wild-type

Received 22 November 2022; revised 22 June 2023; accepted 25 June 2023; accepted manuscript published online 22 July 2023

scores were evaluated, and serum IgE levels were measured in patients with AD. The results showed that there was no significant association between IgE levels and AD severity (Figure 1a). Serum IL-4, IL-13, and TSLP levels were significantly higher in patients with AD than in healthy controls (Figure 1b). Furthermore, no significant correlation was found between IgE and type 2 cytokines (such as IL-4, IL-13, and TSLP) in patients with AD (Figure 1c). In addition, the results in MC903-treated AD-like mice were consistent with those in patients with AD. Compared with the control group, the Atopic Dermatitis Index (ADI) score, scratching bouts, redness, dryness, and lichenification were obvious in the

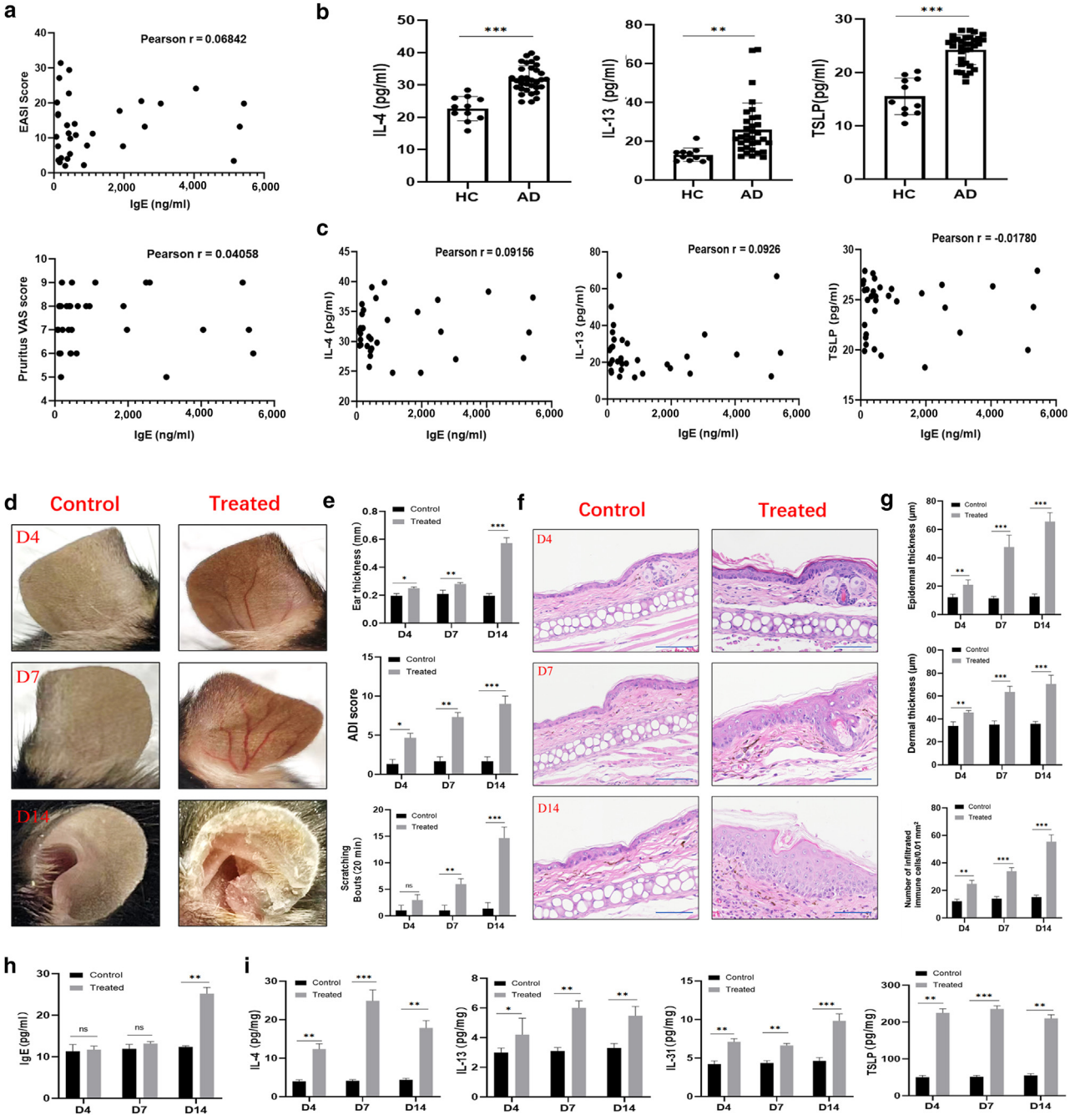


Figure 1. AD severity and type 2 cytokine release were not strongly associated with IgE levels. (**a**) Analysis of the association between IgE levels in patients with AD and their EASI scores and pruritus VAS score (n = 33). (**b**) Determination of serum IL-4, IL-13, and TSLP levels in patients with AD and HCs (n = 11, n = 33 for AD). (**c**) Analysis of the association between IgE levels in patients with AD and their serum IL-4, IL-13, and TSLP levels (n = 33). (**d**) Phenotypes in the ears of mice with AD and the controls. (**e**) Ear thickness, ADI score, and scratching times in 20 min for mice with AD and the controls. (**f**) Histopathological results of mice with AD and the controls. Bars = 50 μm. (**g**) Epidermal thickness, dermal thickness, and the number of infiltrated immune cells in mice with AD and the controls. (**h**) Serum IgE level between MC903-treated mice with AD and the controls on day 4, day 7, and day 14. (**i**) IL-4, IL-13, IL-31, and TSLP levels of ear tissue in mice with AD and the controls (n = 6). Each data point represents an individual. Differences were considered significant at $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. D4, D7, and D14 denote days 4, 7, and 14, respectively. AD, atopic dermatitis; ADI, Atopic Dermatitis Index; EASI, Eczema Area and Severity Index; HC, healthy control; min, minute; ns, no significant; TSLP, thymic stromal lymphopoietin; VAS, Visual Analog Scale.

MC903-treated group (Figure 1d and e). Microscopy analysis showed that both epidermal and dermal thickness increased in the MC903-treated group. Inflammatory infiltration (mainly lymphocytes, neutrophils, and several eosinophils) with vasodilation was observed in the dermis of the MC903-treated group compared with that in the control group, which showed negligible symptoms (Figure 1f and g). Notably, type 2 cytokine (IL-4, IL-13, or IL-31) and TSLP levels increased from day 4 (Figure 1i), whereas no significant elevations in IgE levels were detected in MC903-treated mice with AD until day 14 (Figure 1h), indicating that in the early stages of AD without elevated IgE, the inflammatory phenotype is already present, and type 2 factors are already released.

MC activation and elevated MRGPRX2 expression correlate with type 2 cytokines release in AD

To define the role of MCs in AD, toluidine blue and avidin staining in AD skin lesions was used to first detect MCs, which showed an increased number of MCs with degranulation in the dermis of patients with AD and in the MC903-treated AD mouse model (Figure 2a, b, and d). Furthermore, MRGPRX2 expression during AD inflammation was measured. ELISA and immunohistochemical staining of blood and tissue samples from patients with AD showed increased MRGPRX2 in these samples compared with those from healthy controls (Figure 2a–c and e). Furthermore, we found that MRGPRX2 levels were positively correlated with IL-4, IL-13, TSLP, substance P, and calcitonin gene-related

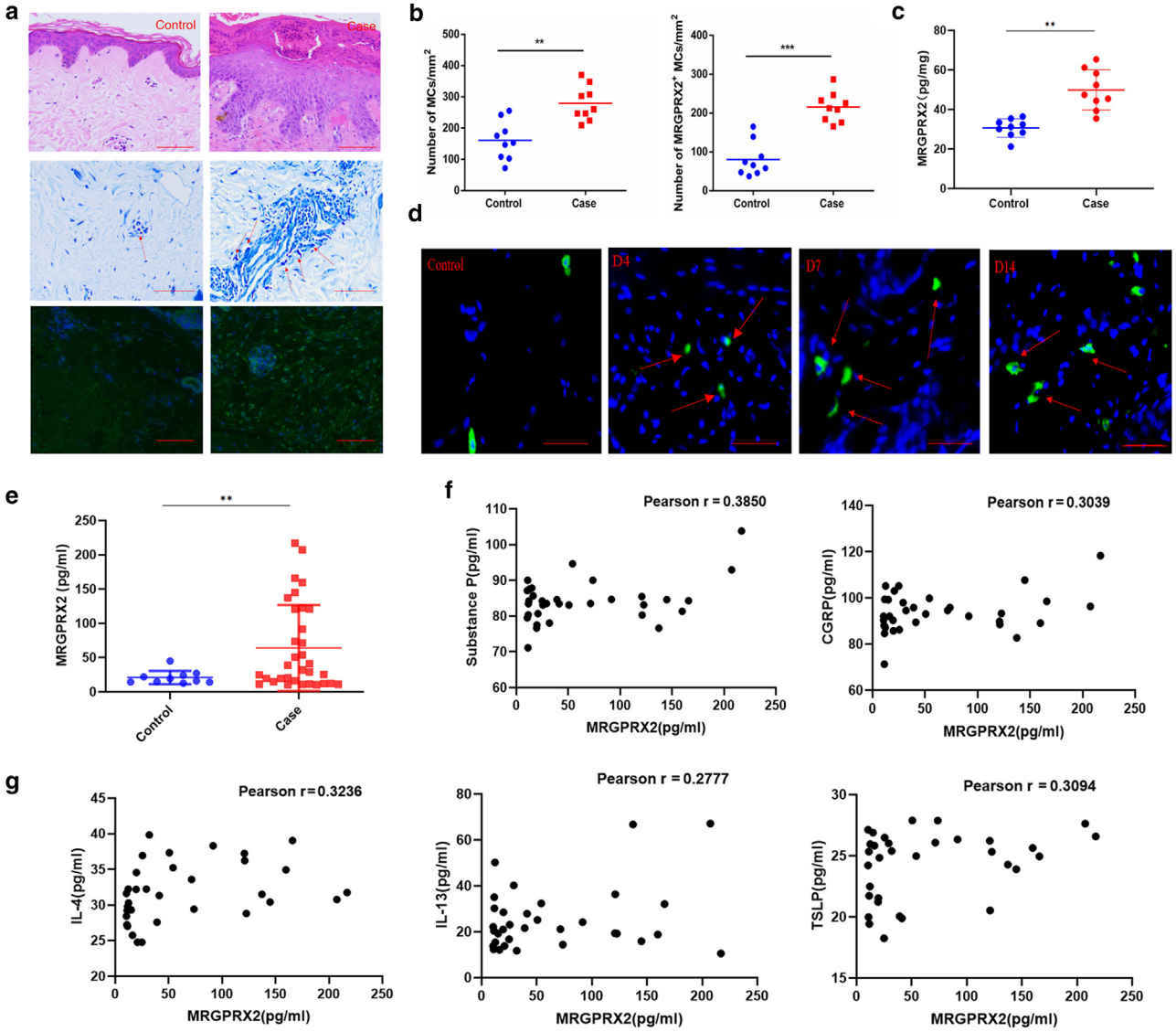


Figure 2. Mast cell activation and elevated MRGPRX2 expression correlate with type 2 cytokines release in AD. (**a**) H&E staining, toluidine blue staining, and immunofluorescence staining of MRGPRX2 of skin lesions of patients with AD and healthy controls. Arrows point to MCs. (**b**) The number of MCs and MRGPRX2$^+$ MCs in skin lesions of patients with AD and healthy controls. (**c**) MRGPRX2 levels in lesions of patients with AD and healthy controls (n = 9). (**d**) Immunofluorescence staining of avidin in ear skin lesions of mice with AD on day 4, day 7, and day 14. Bars = 50 μm; arrows point to MCs. (**e**) MRGPRX2 levels in blood samples of patients with AD and healthy controls (control, n = 11; cases, n = 33). (**f, g**) The correlation of blood MRGPRX2 levels and serum IL-4, IL-13, TSLP, SP, and CGRP in patients with AD (each data point represents an individual). Differences were considered significant at $^{**}P < 0.01$ and $^{***}P < 0.001$. AD, atopic dermatitis; CGRP, calcitonin gene-related peptide; MC, mast cell; SP, substance P; TSLP, thymic stromal lymphopoietin.

peptide in patients with AD (Figure 2f and g). Notably, substance P and calcitonin gene-related peptide are important itching-inducing mediators of pruritus in chronic pruritus dermatitis (Choi and Di Nardo, 2018). Moreover, we determined the relationship between MC activation and type 2 inflammation in AD. Type 2 cytokine increased from day 4 after MC903 AD treatment (Figure 1i); however, elevated IgE was not observed until day 14 (Figure 1h), implying that MC activation is associated with type 2 cytokine release in an IgE-independent manner in the early stages of AD.

MCs mediate the phenotypic regulation and type 2 cytokines release in AD

To further validate the role of MC activation in AD, an MC903-treated AD model was constructed using Kit$^{W\text{-}sh/W\text{-}sh}$ mice (MC deficient), which showed reduced dermatitis, inflammatory infiltration, and decreased type 2 cytokine and TSLP release compared with MC903-treated wild-type (WT) mice. No significant difference was observed between WT control mice and Kit$^{W\text{-}sh/W\text{-}sh}$ control mice (Figure 3a–e). Furthermore, an increased number of MCs were detected in MC903-treated mice with AD using toluidine blue staining (Figure 3f). Examination of the signaling pathways in WT mice with AD using western blotting revealed that the downstream PLCγ1 signaling pathway of MRGPRB2 was activated by phosphorylation in WT mice with AD; however, the downstream Lyn signaling pathway of IgE was not activated (Figure 3g). In addition, in *MrgprB2*$^{-/-}$ mice with AD, phosphorylation levels of both PLCγ1 and Lyn signaling pathways were not significantly increased (Figure 3h). These data further suggest that MCs are involved in early AD pathogenesis through MRGPRX2 activation.

MRGPRX2 is involved in AD inflammation and type 2 cytokine release mediated by MCs

To confirm the key role of MRGPRX2 in AD inflammation and type 2 cytokine release, an MC903-treated AD model was established using *MrgprB2*-conditional knockout

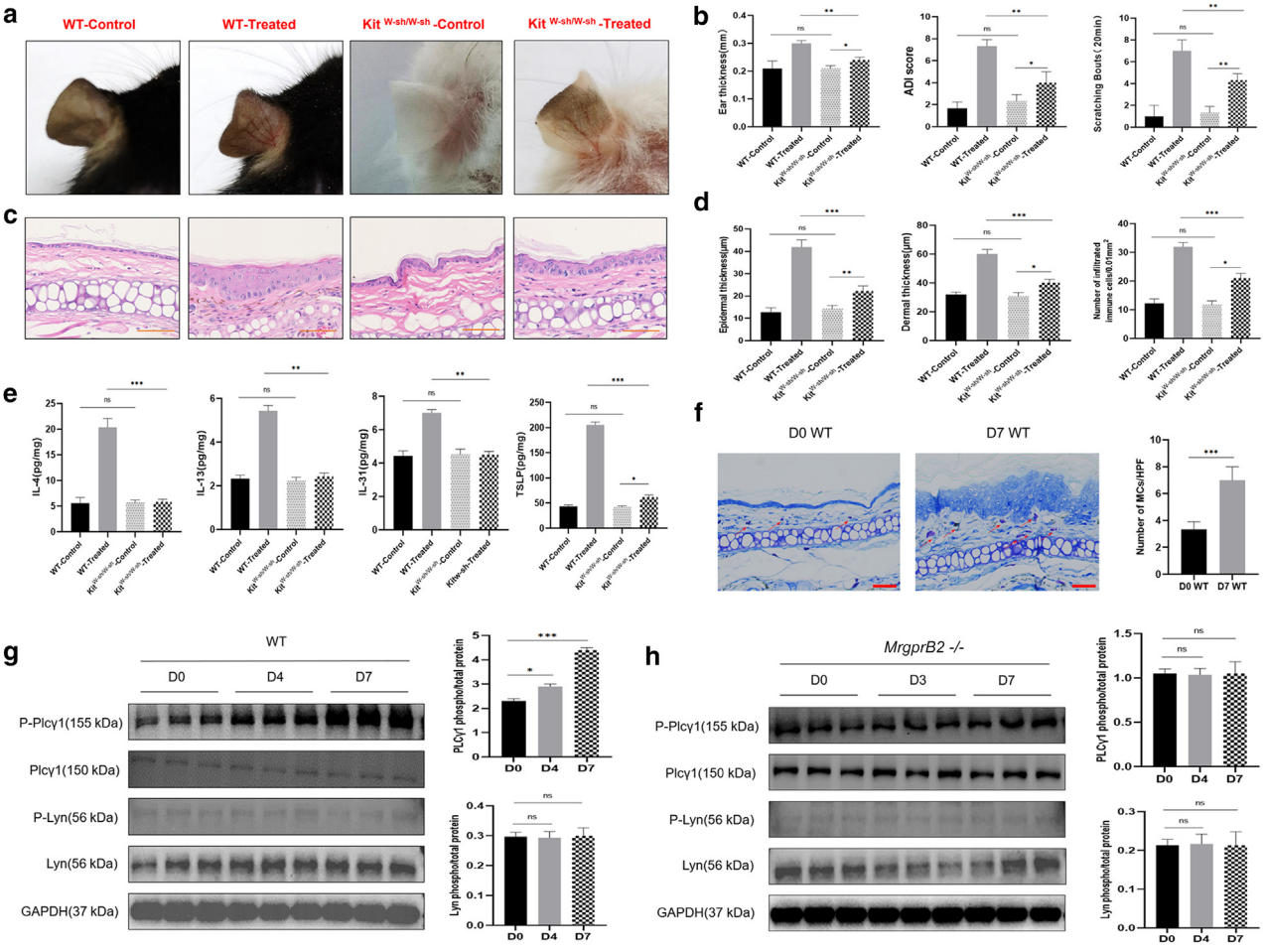


Figure 3. MCs mediate the phenotypic regulation and type 2 cytokines release in AD. (**a**) Phenotypes of the ears of MC-deficient Kit$^{W\text{-}sh/W\text{-}sh}$ mice with AD and WT mice with AD on day 7. (**b**) Ear thickness, ADI score, and scratching times of MC-deficient Kit$^{W\text{-}sh/W\text{-}sh}$ mice with AD and WT mice with AD (n = 6). (**c**) Histopathological results of MC-deficient Kit$^{W\text{-}sh/W\text{-}sh}$ mice with AD and WT mice with AD. Bars = 50 μm. (**d**) Epidermal thickness, dermal thickness, and the number of infiltrated immune cells of MC-deficient Kit$^{W\text{-}sh/W\text{-}sh}$ mice with AD and WT mice with AD (n = 6). (**e**) IL-4, IL-13, IL-31, and TSLP of ear tissues of MC-deficient Kit$^{W\text{-}sh/W\text{-}sh}$ mice with AD and WT mice with AD on day 7 (n = 6). (**f**) Toluidine blue staining of MC903-treated WT mice (n = 6, arrows point to MCs). (**g**) Detection of downstream signaling pathways in the MC903-treated WT mice by western blotting. (**h**) Detection of downstream signaling pathways in the MC903-treated *MrgprB2*$^{-/-}$ mice by western blotting (each data point represents an individual). Differences were considered significant at $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. D0, D3, D4, and D7 denotes days 0, 3, 4, and 7, respectively. AD, atopic dermatitis; ADI, Atopic Dermatitis Index; MC, mast cell; ns, no significant; p-Lyn, phosphorylated Lyn; p-Plcγ1, phosphorylated Plcγ1; TSLP, thymic stromal lymphopoietin; WT, wild-type.

(*MrgprB2*$^{-/-}$) mice. After topical application of MC903 to WT and *MrgprB2*$^{-/-}$ mice, the mouse phenotype and scratching behavior were observed. The ADI score, scratching bouts, redness, dryness, and lichenification decreased in the *MrgprB2*$^{-/-}$ group compared with those in the WT group (Figure 4a and c). Furthermore, histopathological observations showed a decrease in epidermal thickness, dermal thickness, and inflammatory infiltration in the dermis of the *MrgprB2*$^{-/-}$ group compared with those in the WT group (Figure 4b and d). Notably, there was no significant difference in the phenotype or inflammatory infiltration between WT control mice and *MrgprB2*$^{-/-}$ control mice. However, elevated serum IgE was found on day 14 in *MrgprB2*$^{-/-}$ mice compared with that in WT mice (Figure 4e), which confirmed an absence of correlation between MRGPRB2 and IgE in AD inflammation. The levels of type 2 cytokines—IL-4, IL-13, IL-31, and TSLP—were significantly decreased in the *MrgprB2*$^{-/-}$ group compared with those in the WT group on days 4, 7, and 14, whereas there were no significant differences between WT control and *MrgprB2*$^{-/-}$ control mice (Figure 4f and g). Collectively, these results strongly suggest that activated MRGPRX2 in MCs is correlated with AD inflammation, particularly for type 2 cytokines.

MRGPRX2/MRGPRB2—tryptase axis contributes to AD phenotype and type 2 cytokines release

The mechanism by which MRGPRX2/MRGPRB2 mediates type 2 inflammation should be explored further. We found that tryptase specifically released by MCs was significantly higher in patients with AD than in healthy controls (Figure 5a), and serum tryptase concentrations in patients with AD were positively correlated with MRGPRX2 levels (Figure 5b). In the MC903-treated AD mouse model, tryptase was elevated in WT mice but decreased in *MrgprB2*$^{-/-}$ mice (Figure 5c). Furthermore, the mild inflammatory phenotype and infiltration in MC903-treated *MrgprB2*$^{-/-}$ mice were relieved by the topical application of tryptase. The application of tryptase antagonists to MC903-treated WT mice resulted in a milder inflammatory phenotype and less inflammatory infiltration than in WT mice treated with MC903 alone. Furthermore, our results revealed that ear thickness, ADI score, scratching frequency, and inflammation infiltration increased significantly after tryptase supplementation compared with those in *MrgprB2*$^{-/-}$ mice treated only with MC903. In contrast, dermatitis and pruritus were reduced by application of a tryptase antagonist in WT mice with AD (Figure 5d–g). To further investigate the effect of the

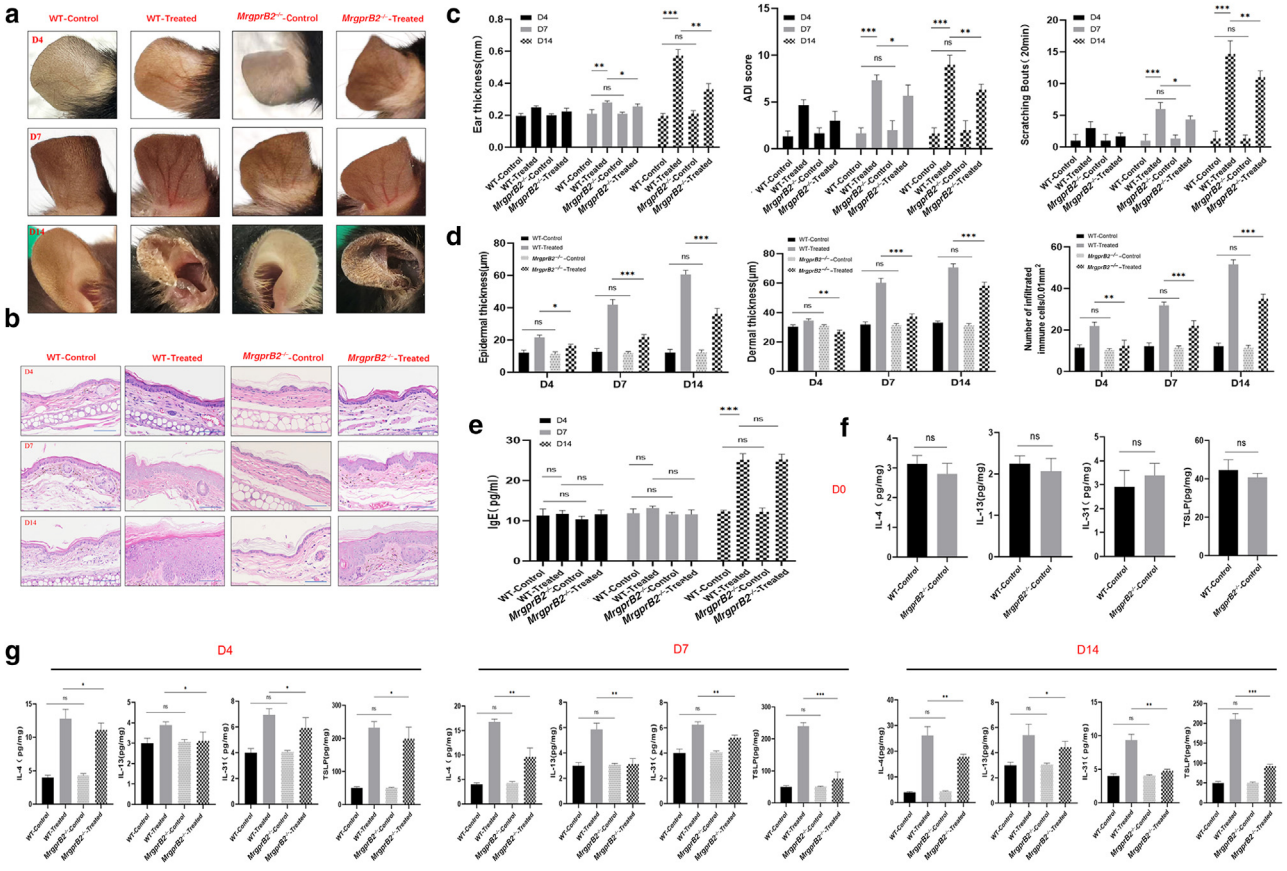


Figure 4. MRGPRX2 is involved in AD inflammation and type 2 cytokine release mediated by mast cells. (**a**) The phenotype of ears in *MrgprB2*$^{-/-}$ MC903-treated mice and WT MC903-treated mice. (**b**) Histopathology results of *MrgprB2*$^{-/-}$ MC903-treated mice and WT MC903-treated mice. Bars = 50 μm. (**c**) The ear thickness, ADI score, and scratching times in 20 min for *MrgprB2*$^{-/-}$ mice with AD and WT mice with AD. (**d**) Epidermal thickness, dermal thickness, and the number of infiltrated immune cells of *MrgprB2*$^{-/-}$ mice with AD and WT mice with AD. (**e**) Serum IgE level of *MrgprB2*$^{-/-}$ mice with AD and WT mice with AD. (**f, g**) IL-4, IL-13, IL-31, and TSLP concentration in ear tissues of *MrgprB2*$^{-/-}$ mice with AD and WT mice with AD on day 0, day 4, day 7, and day 14 (n = 6). Differences were considered significant at $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. D0, D4, D7, and D14 denotes days 0, 4, 7, and 14, respectively. AD, atopic dermatitis; ADI, Atopic Dermatitis Index; min, minute; ns, no significant; TSLP, thymic stromal lymphopoietin; WT, wild-type.

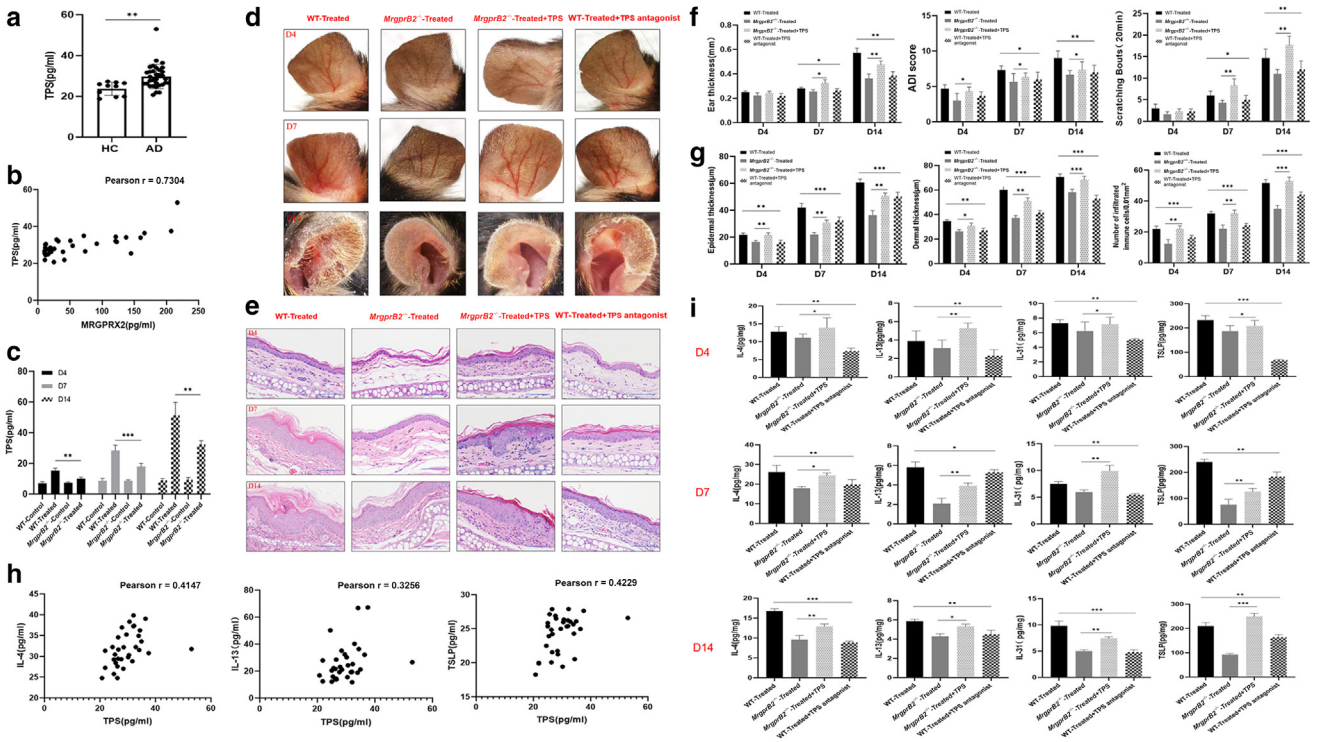


Figure 5. MRGPRX2/MRGPRB2—TPS axis contributes to AD phenotype and type 2 cytokines release. (**a**) Serum TPS concentration of patients with AD and healthy controls (control, n = 11; cases, n = 33). (**b**) The correlation between serum TPS concentration and MRGPRX2 levels in patients with AD (n = 33). (**c**) Serum TPS concentration in the WT mice with AD and *MrgprB2*$^{-/-}$ mice with AD (n = 6). (**d**) The phenotype of ears of WT-treated mice, *MrgprB2*$^{-/-}$-treated mice, *MrgprB2*$^{-/-}$-treated mice with TPS application, and WT-treated mice with TPS antagonist gavage. (**e**) Ear skin histological results of WT-treated mice, *MrgprB2*$^{-/-}$-treated mice, *MrgprB2*$^{-/-}$-treated mice with TPS application, and WT-treated mice with TPS antagonist gavage. Bars = 50 μm. (**f**) Ear thickness, ADI score, and scratching bouts in 20 min for WT-treated mice, *MrgprB2*$^{-/-}$-treated mice, *MrgprB2*$^{-/-}$-treated mice with TPS application, and WT-treated mice with TPS antagonist gavage (n = 6). (**g**) Epidermal thickness, dermal thickness, and the number of infiltrated immune cells in WT-treated mice, *MrgprB2*$^{-/-}$-treated mice, *MrgprB2*$^{-/-}$-treated mice with TPS application, and WT-treated mice with TPS antagonist gavage (n = 6). (**h**) The correlation of serum IL-4, IL-13, and TSLP concentration and serum TPS concentration of patients with AD (n = 33). (**i**) IL-4, IL-13, IL-31, and TSLP concentration in ear tissue of WT-treated mice, *MrgprB2*$^{-/-}$-treated mice, *MrgprB2*$^{-/-}$-treated mice with TPS application, and WT-treated mice with TPS antagonist gavage (n = 6). Differences were considered significant at $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. D4, D7, and D14 denotes days 4, 7, and 14, respectively. AD, atopic dermatitis; ADI, Atopic Dermatitis Index; min, minute; TPS, tryptase; TSLP, thymic stromal lymphopoietin; WT, wild-type.

MRGPRX2/MRGPRB2—tryptase axis on AD, type 2 cytokines were measured in patients with AD and in mice with AD. A positive correlation was observed between serum tryptase concentrations and type 2 cytokine levels, including IL-4, IL-13, and TSLP, in patients with AD (Figure 5h). In addition, IL-4, IL-13, IL-31, and TSLP levels were significantly increased after tryptase supplementation in *MrgprB2*$^{-/-}$ mice with AD and were reduced after tryptase antagonist application in WT mice with AD (Figure 5i). In summary, it was concluded that MRGPRX2/MRGPRB2 is involved in the regulation of type 2 inflammation in AD through tryptase.

Tryptase could lead to type 2 inflammation in vivo

To further investigate the relationship between tryptase and type 2 inflammation, tryptase and saline were applied separately to the ear skin of WT mice. Phenotypes were observed on days 4, 7, and 14. Ears of tryptase-treated mice were found to be significantly more reddish, swollen, dry, and flaky than those of saline-treated mice. The ear thickness, ADI score, and scratching bouts were also increased in tryptase-treated mice (Figure 6a and c). Histopathological findings showed that mice in the tryptase-treated group had increased epidermal and dermal thickness and more severe inflammatory infiltration than those in the control group (Figure 6b and d). Furthermore, IL-4, IL-13, IL-31, and TSLP levels in tissues were significantly elevated in tryptase-treated mice compared with those in the controls (Figure 6e). In conclusion, it was demonstrated that tryptase contributes to type 2 inflammation in vivo.

DISCUSSION

Atopy indicates the frequent, concomitant occurrence of IgE-mediated hypersensitivity reactions (Ring, 2014). Disruption of the epidermal barrier and activation of epidermal dendritic and innate lymphoid cells can initiate inflammation in AD. Most studies have reported an increase in the number of MCs in AD, which was reconfirmed by this study. Notably, MCs bridge innate and adaptive immunity (Taube and Stassen, 2008). Furthermore, MCs act as storehouses in the skin for many biologically active and immunomodulatory molecules, for example, tryptase, multiple chemokines, cytokines, and histamines (Metcalfe et al., 1997; Wernersson and Pejler, 2014). High-affinity FcεRI on MCs can cross-link antigen-specific IgE to trigger the release of mediators, such as histamines, cytokines, and proteases, to drive allergic reactions and pruritus (Amin, 2012; Hellman et al., 2017).

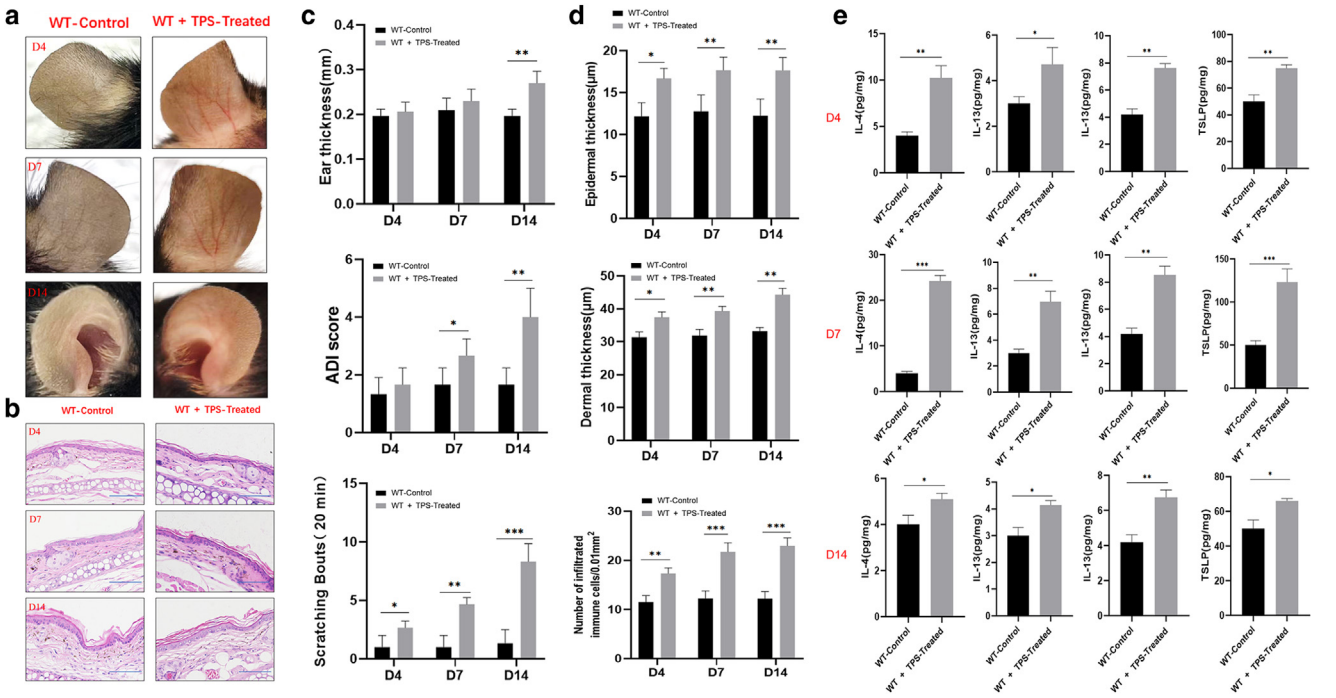


Figure 6. TPS could lead to type 2 inflammation in vivo. (**a**) The phenotype of TPS-treated WT mice and saline-treated WT mice. (**b**) Histopathological results of TPS-treated WT mice and the controls. Bars = 50 μm. (**c**) Ear thickness, ADI score, and scratching bouts in 20 min for TPS-treated WT mice and the controls. (**d**) Epidermal thickness, dermal thickness, and the number of infiltrated immune cells of TPS-treated WT mice and the controls. (**e**) IL-4, IL-13 IL-31, and TSLP of ear tissue of TPS-treated WT mice and the controls (n = 6). Differences were considered significant at $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$. D4, D7, and D14 denotes days 4, 7, and 14, respectively. AD, atopic dermatitis; ADI, Atopic Dermatitis Index; min, minute; TPS, tryptase; TSLP, thymic stromal lymphopoietin; WT, wild-type.

However, in clinical practice, elevated serum IgE is not recommended in many guidelines for AD diagnosis or as a routine monitoring index of disease severity (Eichenfield et al., 2014; Katoh et al., 2020; Ständer, 2021). This study also showed that serum IgE levels were not correlated with pruritus score and Eczema Area and Severity Index score in patients with AD, among whom approximately 30% had normal IgE levels. In addition, the poor outcome of antihistamines and omalizumab treatment in patients with AD suggests that IgE is not a vital component in AD development (Erickson et al., 2018; Frazier and Bhardwaj, 2020; Heil et al., 2010; LePoidevin et al., 2019; Siegels et al., 2021). Some studies showed that MRGPRX2/MRGPRB2 can be stimulated to activate and degranulate MCs, releasing mediators that differ from those of the classical IgE pathway and contribute to chronic itching (Gaudenzio et al., 2016; McNeil et al., 2015; Takamori et al., 2019).

An AD-like mouse model was constructed using MC903 topical application to further investigate the role and mechanism of MRGPRX2/MRGPRB2 in AD pathogenesis. Notably, serum IgE levels were elevated until day 14, whereas type 2 cytokine levels were elevated from day 4 after MC903 treatment, suggesting that type 2 cytokines were activated before IgE production. The key role of T helper 2 cells in AD pathogenesis has been established previously. Dysregulation of T helper 2 cells and the release of type 2 cytokines, such as IL-4, IL-13, IL-31, and TSLP, promote eczematous lesions (Ständer, 2021). However, the mechanisms that initiate inflammation in the early stages of AD have not yet been elucidated. Notably, TSLP from activated keratinocytes stimulates type 2 cytokine production (Allakhverdi et al., 2007; Jariwala et al., 2011; Mizutani et al., 2015). Furthermore, MC-specific tryptase proteases have been reported to activate keratocytes to release TSLP and drive type 2 inflammation through the PAR2 receptor in vitro (Redhu et al., 2022). However, whether keratinocytes are triggered by MC tryptase to release TSLP and subsequent type 2 inflammation in AD has not been verified yet.

Tryptase is an important inflammatory mediator released by MCs through MRGPRX2 activation. In a study on chronic pruritus, MRGPRX2 was found to be highly expressed in pruritic AD lesions by RNA sequencing, and degranulated MCs could drive house dust mite—induced type 2 skin inflammation by releasing substance P and activating MRGPRB2 (Nattkemper et al., 2018; Serhan et al., 2019). However, the mechanism through which MRGPRX2 drives type 2 inflammation in patients with AD has not been explained in detail. This study observed an increase in MRGPRX2 levels in the lesions and blood of patients with AD and found increased MC activation and release of MC mediators, including tryptase, in early skin lesions in an AD mouse model. Moreover, MRGPRX2 expression was correlated with serum IL-13 and TSLP levels in patients with AD. In *MrgprB2*$^{-/-}$ mice with AD, activation of MCs through MRGPRX2/MRGPRB2 receptors was found to regulate type 2 inflammation.

Moreover, topical application of tryptase to the skin of WT mice resulted in a more severe phenotype and elevated tissue

levels of type 2 cytokines compared with those in untreated WT mice, indicating that tryptase stimulated nonhistamine-dependent pruritus in inflammatory dermatitis. Most studies have focused on the role of tryptase in AD pruritus; however, its effects on AD barrier disruption and type 2 inflammation have not been studied extensively. In tryptase supplementation and suppression experiments, the effects of the tryptase antagonist are most significant on day 4, whereas the differences in the *MrgprB2*$^{-/-}$ mice are more robust on day 14. This may be due to the greater role of IgE in the later stages of the MC903-treated AD mouse model and the interaction between tryptase and IgE pathways, which is involved in the inflammation of AD, but the exact mechanism remains to be further explored (Nguyen et al., 2021). This study's data suggest that the MRGPRB2−tryptase axis is involved in type 2 inflammation in AD and may modulate neuroimmunity in AD.

MC activation can be triggered by the activation of different receptors, such as PAR2 or MRGPRX2, resulting in the release of tryptase rather than histamine or chymase, which then activates keratocytes and triggers inflammation in AD (Meixiong et al., 2019; Redhu et al., 2022). Contrasting with this study, Meixiong et al. (2019) reported no reduction in scratching bouts after complete knockdown of *MrgprB2*. This difference may be because of the different structures of the mice because their study involved the construction of mice with complete deletion of *MrgprB2*, which may affect the function of some nerve cell basophils and eosinophils, leading to abnormalities in the production of itching sensations. In contrast, in this study, *Cpa3*-cre; *MrgprB2*-flox/flox mice were MC-specific conditional *MrgprB2*-knockout mice constructed by the Cre-Loxp recombinase system, which resulted in better MC specificity and prevented any effect of other cells expressing the MRGPRB2 receptor compared with Mrgprb2 fully knockout mice.

MC activation through MRGPRB2 combines with TRPV1^{+}Tac1^{+} nociceptors to release neuropeptides and induce pruritus in AD (Serhan et al., 2019). It has been indicated that MCs can regulate epidermal barrier function in allergic skin inflammation, though the specific mechanism has not yet been elucidated (Sehra et al., 2016). Therefore, the role of intrinsic immune cells, such as MCs, in early AD is not negligible, and such cells may also play an important regulatory role in the neuroimmune network. The cross-linking and specific mechanisms of MCs with keratocytes, T helper 2 cells, and other lymphocytes in AD are worth exploring.

In conclusion, this study demonstrated that the activation of MRGPRX2 could affect chronic pruritus in AD and initiate type 2 cytokines. Furthermore, it was found that tryptase released from MCs contributed to the AD phenotype and type 2 inflammation in an AD mouse model. These data suggest that the MRGPRX2/MRGPRB2−tryptase axis is crucial for the early development of inflammation in AD (Supplementary Figure S1). It may be worthwhile to address the role of the MRGPRX2/MRGPRB2−tryptase axis in the neuroimmune mechanism of AD. In addition, MRGPRX2 may be, to our knowledge, a potential previously unreported therapeutic target for treatment of AD.

MATERIALS AND METHODS

Study approval

This study was registered at the Chinese Clinical Trial Registry, and the registration number is ChiCTR2200063267. The ethical approval (2022008) was provided by Ethics Committee at The Second Affiliated Hospital of Xi'an Jiaotong University (Xi'an, China) and conformed to the ethical standard. All the specimens in this study had signed informed consent. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals from the National Institutes of Health.

Patients with AD

A single-center and case-control study was adopted. Skin samples from 9 patients and blood samples from 33 patients, who were diagnosed with AD according to Williams diagnosis standards and without other diseases, were from the Department of Dermatology, Second Affiliated Hospital of Xi'an Jiaotong University. Skin control samples from nine patients, who had fractures with no infection and immune diseases, were from the Department of Orthopedics, Second Affiliated Hospital of Xi'an Jiaotong University. Blood control samples were from 11 patients from the Physical Examination Center, Second Affiliated Hospital of Xi'an Jiaotong University.

Mice

Adult male C57BL/6 mice aged 8 weeks were purchased from the Experimental Animal Center of Xi'an Jiaotong University. *MrgprB2*-knockout mice were established on the basis of Cre-floxp recombinase system. C57BL/6 *Cpa3*-cre mice (stock number 026828) were purchased from The Jackson Laboratory (Bar Harbor, ME). *MrgprB2* floxp-floxp mice were provided as a kind gift from the laboratory of Xinzhong Dong at Johns Hopkins University (Baltimore, MD). *MrgprB2*-conditional knockout *Cpa3*-cre; *MrgprB2*-flox/flox (*MrgprB2*$^{-/-}$) mice were acquired by hybridization and backcross. The genotype was verified by RT-qPCR. Six mice were used per group. C57BL/6 STOCK Kit$^{W\text{-}sh}$/HNihrJaeBsmJNju (Kit$^{W\text{-}sh/W\text{-}sh}$) mice were purchased from the Model Animal Research Centre of Nanjing University (Nanjing, China). The mice were housed in a specific pathogen-free animal center in Xi'an Jiaotong University with 20−25 °C, relative humidity of 40%, a day−night cycle of 12/12 hours, and free access to water and were fed standard dry food. All animal experiments involving equal treatments were conducted by researchers who were blinded to the conditions.

AD mouse model

MC903 (calcipotriol, HY-10001) was purchased from MedChemExpress (Shanghai, China). MC903 was prepared with 100% ethanol to a concentration of 45 μM. Male C57BL/6 mice aged 8 weeks were anesthetized with an intraperitoneal injection of 80 mg/kg 1% pentobarbital sodium. Mice were topically applied 12.5 μl MC903 on both ventral and dorsal ear skin once a day for 14 consecutive days. As vehicle control, the same volume of ethanol was applied (Moosbrugger-Martinz et al., 2017).

Tryptase supplementation and suppression experiments

Tryptase beta-2 (HY-P71131) and benzamidine hydrochloride (tryptase antagonist, HY-W018781) were purchased from MedChemExpress. Tryptase was prepared by normal saline to the concentration of 10 μM. Tryptase antagonist was prepared by normal saline to the concentration of 100 μM. In tryptase supplementation and suppression experiments, 20 μl tryptase was applied on the ear skin of *MrgprB2*$^{-/-}$ mice with AD for 14 consecutive days. Tryptase antagonist was applied at 200 μl by intragastric administration on

WT mice with AD for 14 consecutive days. As vehicle control, the same volume of normal saline was applied.

Mice inflammatory phenotype assessment

Mice inflammatory phenotype was assessed by ADI. ADI score was evaluated on the basis of redness, drying, swelling, and lichenification ranked 0, 1, 2, and 3, and the total score is the sum of these scores (Suzuki et al., 2020). The final score is determined by averaging the scores of three trained testers.

While mice were anesthetized, ear thickness was measured with a digital caliper on each treatment day just before the topical application of MC903.

Scratching behavior assessment

The mice were acclimatized in an observation box for 30 minutes at 2 hours after the experimental endpoint and recorded with a video camera for 20 minutes. Scratching behavior was assessed and quantified by trained experimenters from video observation of the relevant treatment groups.

Histology and immunofluorescence staining

Skin samples were fixed in 4% paraformaldehyde and embedded in paraffin. Tissue sections (5 mm) were stained with H&E and were stained with toluidine blue. H&E-stained tissue was evaluated at ×20 magnification. Toluidine blue staining was used to label MCs in tissues. MRGPRX2 was marked with MRGPRX2 Polyclonal Antibody by immunofluorescence staining. The degranulated status of MCs in mice ear tissue was shown by immunofluorescence staining of avidin (the primary antibodies used are detailed in Supplementary Table S1). After tissue sections were dried at 37 °C for 30 minutes and preincubated in blocking solution (10% normal goat serum [vol/vol] and 0.2% Triton X-100 [vol/vol] in PBS, pH 7.4) for 2 hours at 25 °C and incubated with 1:500 FITC-avidin for 45 minutes, sections were washed three times with PBS, and a drop of Fluoro-mount G (Southern Biotech, Birmingham, Alabama) was added. Images were taken immediately with a confocal scanning laser microscope (Nikon, Tokyo, Japan).

Image acquisition, quantitative analysis, ELISA, and MC activation signaling pathways detected by western blotting, data visualization, and statistics

Details are provided in the Supplementary Materials and Methods.

Data availability statement

No datasets were generated or analyzed during this study, and the data that support the findings of this study are available from the correspondence author SG (gengsongmei73@163.com).

ORCIDs

Tao Jia: http://orcid.org/0000-0003-0536-966X
Delu Che: http://orcid.org/0000-0003-1403-7616
Yi Zheng: http://orcid.org/0000-0002-4877-4289
Huan Zhang: http://orcid.org/0000-0001-9816-1192
Yaxiang Li: http://orcid.org/0000-0002-8411-419X
Tong Zhou: http://orcid.org/0000-0003-3056-474X
Bin Peng: http://orcid.org/0000-0001-8489-5726
Xueshan Du: http://orcid.org/0000-0001-7140-2259
Longfei Zhu: http://orcid.org/0000-0001-5647-3067
Jingang An: http://orcid.org/0000-0002-9614-0280
Songmei Geng: http://orcid.org/0000-0001-8731-6267

CONFLICT OF INTEREST

All authors state no conflict of interest.

ACKNOWLEDGMENT

The authors are grateful to Xinyue Zhang, Cong Yan, and Xiaona Li for their contribution to collecting blood and tissue specimens from patients with atopic dermatitis. This work was supported by the National Natural Science Foundation of China (82273526, number 82073451), the Innovation Capability Support Program of Shaanxi (program number 2022TD-48), and the Bethune Charitable Foundation (number 2022HXKT023).

AUTHOR CONTRIBUTIONS

Conceptualization: TJ, DC; Data Curation: TJ, DC, YZ, YL, LZ, BP; Formal Analysis: TJ, DC, HZ, XD; Funding Acquisition: SG, JA; Investigation: TJ, DC, YZ; Methodology: TJ, DC, SG, YZ, HZ, TZ, BP; Project Administration: SG; Resources: SG, JA; Supervision: SG, JA, DC; Validation: TJ, TZ, HZ, BP, LZ; Visualization: TJ, DC, YZ, SG; Writing - Original Draft Preparation: TJ, DC, SG; Writing - Review and Editing: TJ, DC, SG

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.2023.06.201.

REFERENCES

Allakhverdi Z, Comeau MR, Jessup HK, Yoon BR, Brewer A, Chartier S, et al. Thymic stromal lymphopoietin is released by human epithelial cells in response to microbes, trauma, or inflammation and potently activates mast cells. J Exp Med 2007;204:253–8.

Amin K. The role of mast cells in allergic inflammation. Respir Med 2012;106:9–14.

Arents BWM, van Zuuren EJ, Vermeulen S, Schoones JW, Fedorowicz Z. Global Guidelines in Dermatology Mapping Project (GUIDEMAP), a systematic review of atopic dermatitis clinical practice guidelines: are they clear, unbiased, trustworthy and evidence based (CUTE)? Br J Dermatol 2022;186:792–802.

Choi JE, Di Nardo A. Skin neurogenic inflammation. Semin Immunopathol 2018;40:249–59.

Eichenfield LF, Tom WL, Chamlin SL, Feldman SR, Hanifin JM, Simpson EL, et al. Guidelines of care for the management of atopic dermatitis: section 1. Diagnosis and assessment of atopic dermatitis. J Am Acad Dermatol 2014;70:338–51.

Erickson S, Nahmias Z, Rosman IS, Kim BS. Immunomodulating agents as antipruritics. Dermatol Clin 2018;36:325–34.

Frazier W, Bhardwaj N. Atopic dermatitis: diagnosis and treatment. Am Fam Physician 2020;101:590–8.

Gaudenzio N, Sibilano R, Marichal T, Starkl P, Reber LL, Cenac N, et al. Different activation signals induce distinct mast cell degranulation strategies. J Clin Invest 2016;126:3981–98.

Heil PM, Maurer D, Klein B, Hultsch T, Stingl G. Omalizumab therapy in atopic dermatitis: depletion of IgE does not improve the clinical course - a randomized, placebo-controlled and double blind pilot study. J Dtsch Dermatol Ges 2010;8:990–8.

Hellman LT, Akula S, Thorpe M, Fu Z. Tracing the origins of IgE, mast cells, and allergies by studies of wild animals. Front Immunol 2017;8:1749.

Imayama S, Shibata Y, Hori Y. Epidermal mast cells in atopic dermatitis. Lancet 1995;346:1559.

Jariwala SP, Abrams E, Benson A, Fodeman J, Zheng T. The role of thymic stromal lymphopoietin in the immunopathogenesis of atopic dermatitis. Clin Exp Allergy 2011;41:1515–20.

Katoh N, Ohya Y, Ikeda M, Ebihara T, Katayama I, Saeki H, et al. Japanese guidelines for atopic dermatitis 2020. Allergol Int 2020;69:356–69.

Kühn H, Kolkhir P, Babina M, Düll M, Frischbutter S, Fok JS, et al. Mas-related G protein-coupled receptor X2 and its activators in dermatologic allergies. J Allergy Clin Immunol 2021;147:456–69.

Langan SM, Irvine AD, Weidinger S. Atopic dermatitis. Lancet 2020;396:345–60.

LePoidevin LM, Lee DE, Shi VY. A comparison of international management guidelines for atopic dermatitis. Pediatr Dermatol 2019;36:36–65.

McNeil BD, Pundir P, Meeker S, Han L, Undem BJ, Kulka M, et al. Identification of a mast-cell-specific receptor crucial for pseudo-allergic drug reactions. Nature 2015;519:237–41.

Meixiong J, Anderson M, Limjunyawong N, Sabbagh MF, Hu E, Mack MR, et al. Activation of mast-cell-expressed Mas-related G-protein-coupled receptors drives non-histaminergic itch. Immunity 2019;50:1163–71.e5.

Metcalfe DD, Baram D, Mekori YA. Mast cells. Physiol Rev 1997;77:1033–79.

Metzger H. The receptor with high affinity for IgE. Immunol Rev 1992;125:37–48.

Mizutani N, Sae-Wong C, Kangsanant S, Nabe T, Yoshino S. Thymic stromal lymphopoietin-induced interleukin-17A is involved in the development of IgE-mediated atopic dermatitis-like skin lesions in mice. Immunology 2015;146:568–81.

Moosbrugger-Martinz V, Schmuth M, Dubrac S. A mouse model for atopic dermatitis using topical application of vitamin D3 or of its analog MC903. Methods Mol Biol 2017;1559:91–106.

Nattkemper LA, Tey HL, Valdes-Rodriguez R, Lee H, Mollanazar NK, Albornoz C, et al. The genetics of chronic itch: gene expression in the skin of patients with atopic dermatitis and psoriasis with severe itch. J Invest Dermatol 2018;138:1311–7.

Nguyen SMT, Rupprecht CP, Haque A, Pattanaik D, Yusin J, Krishnaswamy G. Mechanisms governing anaphylaxis: inflammatory cells, mediators, endothelial gap junctions and beyond. Int J Mol Sci 2021:22.

Payne V, Kam PC. Mast cell tryptase: a review of its physiology and clinical significance. Anaesthesia 2004;59:695–703.

Redhu D, Franke K, Aparicio-Soto M, Kumari V, Pazur K, Illerhaus A, et al. Mast cells instruct keratinocytes to produce thymic stromal lymphopoietin: relevance of the tryptase/protease-activated receptor 2 axis. J Allergy Clin Immunol 2022;149:2053–61.e6.

Ring J. Terminology of allergic phenomena. Chem Immunol Allergy 2014;100:46–52.

Sehra S, Serezani APM, Ocaña JA, Travers JB, Kaplan MH. Mast cells regulate epidermal barrier function and the development of allergic skin inflammation. J Invest Dermatol 2016;136:1429–37.

Serhan N, Basso L, Sibilano R, Petitfils C, Meixiong J, Bonnart C, et al. House dust mites activate nociceptor-mast cell clusters to drive type 2 skin inflammation. Nat Immunol 2019;20:1435–43.

Siegels D, Heratizadeh A, Abraham S, Binnmyr J, Brockow K, Irvine AD, et al. Systemic treatments in the management of atopic dermatitis: a systematic review and meta-analysis. Allergy 2021;76:1053–76.

Ständer S. Atopic dermatitis. N Engl J Med 2021;384:1136–43.

Suzuki T, Nishiyama K, Kawata K, Sugimoto K, Isome M, Suzuki S, et al. Effect of the *Lactococcus lactis* 11/19-B1 strain on atopic dermatitis in a clinical test and mouse model. Nutrients 2020;12:763.

Svanberg S, Li Z, Öhlund P, Roy A, Åbrink M. Mast cells limit ear swelling independently of the chymase mouse mast cell protease 4 in an MC903-induced atopic dermatitis-like mouse model. Int J Mol Sci 2020;21:6311.

Takamori A, Izawa K, Kaitani A, Ando T, Okamoto Y, Maehara A, et al. Identification of inhibitory mechanisms in pseudo-allergy involving Mrgprb2/MRGPRX2-mediated mast cell activation. J Allergy Clin Immunol 2019;143:1231–5.e12.

Taube C, Stassen M. Mast cells and mast cell-derived factors in the regulation of allergic sensitization. Chem Immunol Allergy 2008;94:58–66.

Thapaliya M, Chompunud Na Ayudhya C, Amponnawarat A, Roy S, Ali H. Mast cell-specific MRGPRX2: a key modulator of neuro-immune interaction in allergic diseases. Curr Allergy Asthma Rep 2021;21:3.

Voss M, Kotrba J, Gaffal E, Katsoulis-Dimitriou K, Dudeck A. Mast cells in the skin: defenders of integrity or offenders in inflammation? Int J Mol Sci 2021;22.

Wernersson S, Pejler G. Mast cell secretory granules: armed for battle. Nat Rev Immunol 2014;14:478–94.

SUPPLEMENTARY MATERIALS AND METHODS

Image acquisition and quantitative analysis

All stained sections are scanned by NDP software (San Francisco, CA) (Hamamatsu Nanozoomer Digital Pathology System) to obtain images with identical conditions of exposure and background balance. Six independent biological replicates were chosen and measured to represent statistical significance between groups. The thickness of the epidermis and the dermis was measured using ImageJ software (National Institutes of Health, Bethesda, MD). Counting of the immune cells was done manually in a defined area of the tissues. Five random areas were selected, and their average represents the individual measurements.

ELISA

Blood and tissue samples of patients with atopic dermatitis and healthy control were collected. Mice ear samples and human tissue samples were ground frozen with liquid nitrogen, and 0.5 ml saline was added. Then, the tissues were ground in a tissue grinder (Servicebio, Wuhan, China). The blood samples were centrifuged to obtain serum. Human MRGPRX2, tryptase, IL-4, IL-13, thymic stromal lymphopoietin, substance P, and calcitonin gene-related peptide ELISA Kit and mouse tryptase, IL-4, IL-13, IL-31, and thymic stromal lymphopoietin ELISA Kit were purchased from Shanghai MLBIO Biotechnology (Shanghai, China), and human IgE and mouse IgE ELISA Kits were purchased from Elabscience (Wuhan, China). Total protein content in tissues was determined using BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA). All steps were performed strictly according to the manufacturer's instructions.

Mast cell activation signaling pathways detected by western blotting

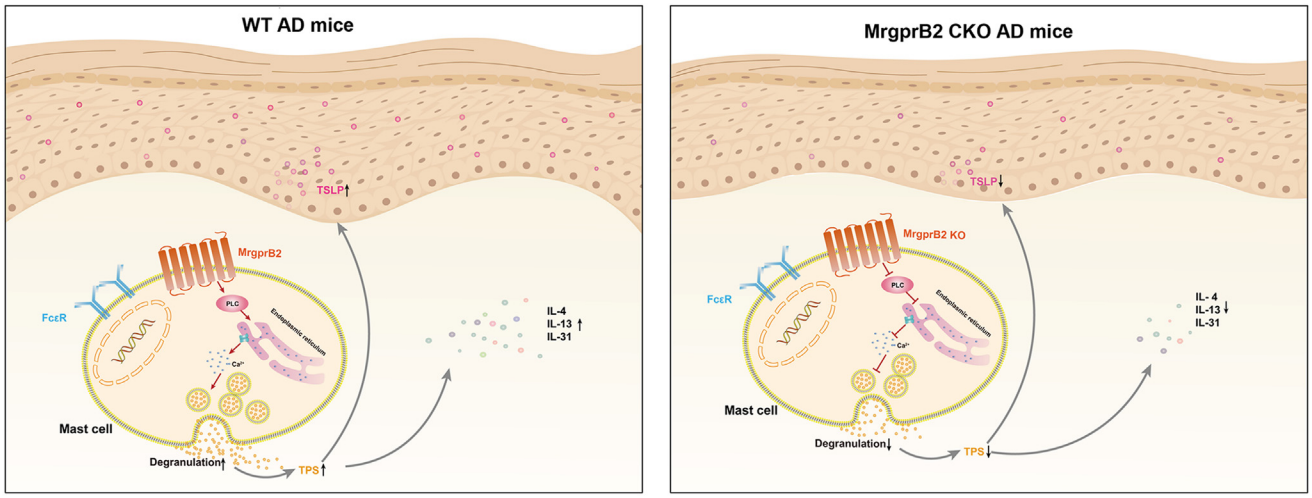
The ear tissue was lysed in RIPA buffer containing a protease inhibitor cocktail and protein phosphatase inhibitors (Roche Diagnostics, Basel, Switzerland). Protein concentrations were determined using the BCA protein assay kit (Thermo Fisher Scientific) and 150 g/lane of proteins were separated by SDS-PAGE and transferred to polyvinylidene fluoride membrane (Hangzhou Microna Membrane Technology, Hangzhou, China). Immunoblotting was done following the procedure described previously (Zheng et al., 2019), and the primary antibodies used are detailed in Supplementary Table S1. Quantification of protein grayscale values was analyzed by ImageJ software (National Institutes of Health).

Data visualization and statistics

All graphical representation of data was done using Prism 8.0 (GraphPad Software, La Jolla, CA). An independent samples analysis of variance was used to determine statistical significance in comparisons of the data using the Statistical Package for the Social Sciences software. Data are expressed as mean ± SEM. For comparison between two groups, Student's *t*-test followed by a Mann–Whitney posthoc test was used. For the paired comparison samples, the treated groups were compared with the negative control group. For the multiple doses' samples, the treated groups compared respectively with the negative control group were calibrated by Bonferroni's test. Differences were considered significant at $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$; ns denotes no significance.

SUPPLEMENTARY REFERENCE

Zheng Y, Che D, Peng B, Hao Y, Zhang X, He L, et al. All-trans-retinoic acid activated mast cells via Mas-related G-protein-coupled receptor-X2 in retinoid dermatitis. Contact Dermatitis 2019;81:184–93.



Supplementary Figure S1. Schematic representation of the involvement of MRGPRB2 in the regulation of AD inflammation and type 2 cytokine release. In an MC903-mediated model of AD-like dermatitis, MRGPRB2 mediates mast cell activation and degranulation, causing TPS release and promoting the release of type 2 cytokines and TSLP. In mast cell *MrgprB2*-knockout mice, reduced mast cell activation led to reduced TPS release, resulting in reduced release of type 2 cytokines and TSLP and reduced inflammation in AD. AD, atopic dermatitis; CKO, conditional knockout; KO, knockout; TPS, tryptase; TSLP, thymic stromal lymphopoietin; WT, wild-type.

Supplementary Table S1. Antibodies Used for IHC and WB Analyses

Antigen	Antibody	Species	Company	Catalog Number	IHC/WB Dilution
MRGPRX2	Polyclonal	Rabbit	Thermo Fisher Scientific	PA5-32931	1:50
Avidin	Monoclonal	Rabbit	Solarbio Life Science	SF065	1:150
GAPDH	Polyclonal	Rabbit	ProteinTech	10494-1-AP	1:10,000
PLCγ1	Monoclonal	Rabbit	Cell Signaling Technology	# 5690S	1:1,000
p-PLCγ1	Monoclonal	Rabbit	Cell Signaling Technology	# 14008S	1:1,000
Lyn	Monoclonal	Rabbit	Cell Signaling Technology	# 2796S	1:1,000
p-Lyn	Polyclonal	Rabbit	Cell Signaling Technology	# 2731S	1:1,000

Abbreviations: IHC, immunohistochemistry; p-Lyn, phosphorylated Lyn; p-PLCγ1, phosphorylated PLCγ1; WB, western blotting.