

Journal Pre-proof

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PII: S0022-202X(23)02422-3

DOI: <https://doi.org/10.1016/j.jid.2023.06.201>

Reference: JID 3928

To appear in: *The Journal of Investigative Dermatology*

Received Date: 22 November 2022

Revised Date: 22 June 2023

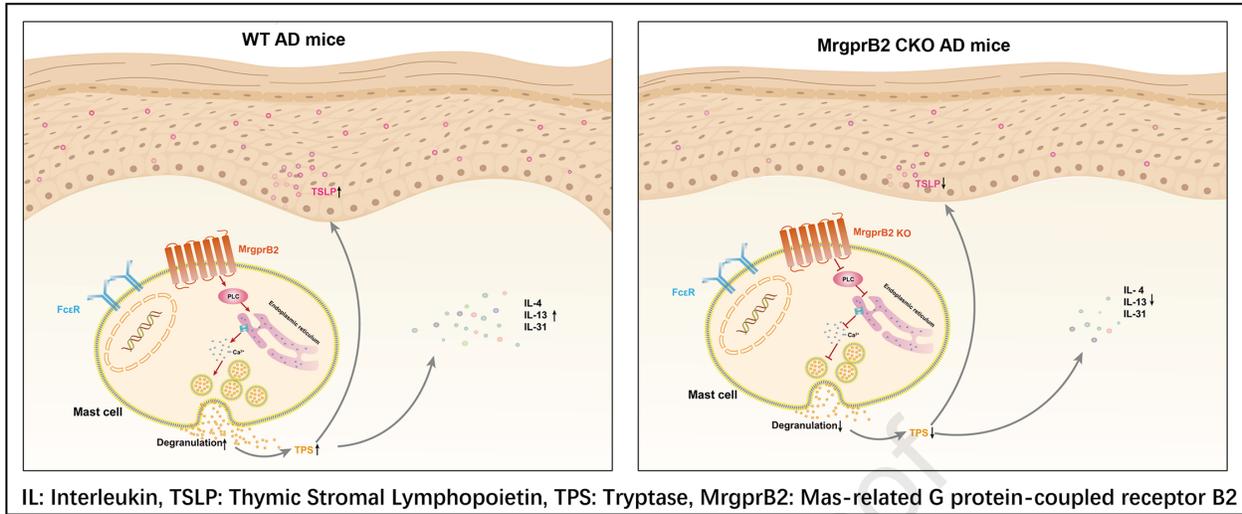
Accepted Date: 25 June 2023

Please cite this article as: Jia T, Che D, Zheng Y, Zhang H, Li Y, Zhou T, Peng B, Du X, Zhu L, An J, Geng S, Mast cells Initiate Type 2 Inflammation via Tryptase Released by MRGPRX2/MrgprB2 Activation in Atopic Dermatitis, *The Journal of Investigative Dermatology* (2023), doi: <https://doi.org/10.1016/j.jid.2023.06.201>.

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Mast cells Initiate Type 2 Inflammation via Tryptase Released by MRGPRX2/MrgprB2

Activation in Atopic Dermatitis

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Short title: Mast cells involved in AD inflammation via MRGPRX2.

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¹**Abbreviations:** AD, atopic dermatitis; Th2, T helper 2 cell; MRGPRX2, Mas-related G-protein-coupled receptor-X2; MrgprB2, Mas-related G-protein-coupled receptor-B2; WT, wild-type; TPS, tryptase; IL, interleukin; TSLP, thymic stromal lymphopoietin; MC, mast cell; SP, substance P; CGRP, calcitonin gene-related peptide; MRGPRs, Mas-related G protein coupled receptors; PAR, Protease-activated receptor; HDM, house dust mites.

ABSTRACT

Atopic dermatitis (AD) is a common chronic inflammatory skin disease characterized by T-helper 2 (Th2) inflammation as the core pathogenic mechanism. MRGPRX2 plays a key role in non-histamine 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 wildtype (WT) 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*^{-/-} AD and WT AD mice 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*^{-/-} AD mice showed milder phenotypes and inflammatory infiltration in the skin than WT AD mice. Tryptase released by MRGPRX2/*MrgprB2* activation is involved in the release of type 2 cytokines, which contribute to the inflammatory development in AD.

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 (Th2)-dominated response is thought to be the inflammatory core of AD, and associated type 2 cytokines include interleukin (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.

Mas-related G protein-coupled receptor-X2 (MRGPRX2) is a multiligand receptor expressed on skin mast cells 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 mast cell granules, is a marker of mast cell 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 Davender et al., 2022).

Determination of whether MRGPRX2/MrgprB2 in mast cells is involved in type 2 inflammation in AD and intractable pruritus via tryptase release is important for 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 (VAS) and eczema area and severity index (EASI) scores were evaluated and serum IgE levels 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 those 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 to the control group, the atopic dermatitis index (ADI) score, scratching bouts, redness, dryness, and lichenification were obvious in the MC903-treated group (Figure 1d, 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 to those in the control group which showed negligible symptoms (Figure 1f, g). Notably, type 2 cytokine (IL-4, IL-13, IL-31) and TSLP levels increased from day 4 (Figure 1i); whereas, no significant elevations in IgE levels were detected in MC903-treated AD mice 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.

Mast cell 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 were 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-AD dermatitis mouse model (Figure 2a-b, 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 than those from healthy controls (Figure 2a-c, e). Furthermore, we found that MRGPRX2 levels were positively correlated with IL-4, IL-13, TSLP, substance P (SP), and calcitonin gene-related peptide (CGRP) in patients with AD (Figure 2f-g). Notably, SP and CGRP are important itching-inducing mediators of pruritus in chronic pruritus dermatitis (Choi and Di Nardo, 2018). Moreover, we determined the relationship between mast cell 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.

Mast cells mediate the phenotypic regulation and type 2 cytokines release in AD

To further validate the role of mast cell activation in AD, an MC903-AD model was constructed using Kit^{W-sh/W-sh} mice (mast cell-deficient), which showed reduced dermatitis, inflammatory infiltration, and decreased type 2 cytokine and TSLP release compared with MC903-WT mice. No significant difference was observed between WT control mice and Kit^{W-sh/W-sh} control mice (Figure 3a-e). Furthermore, an increased number of MCs were detected in MC903-treated AD mice with toluidine blue staining (Figure 3f). Examination of the signaling pathways in WT AD mice using Western Blotting revealed that the downstream PLC γ 1 signaling pathway of

MrgprB2 was activated by phosphorylation in WT AD mice; however, the downstream Lyn signaling pathway of IgE was not activated (Figure 3g). In addition, in MrgprB2^{-/-} AD mice, phosphorylation levels of both PLCγ1 and Lyn signaling pathways were not significantly increased (Figure 3h). These data further suggest that mast cells are involved in early AD pathogenesis through MRGPRX2 activation.

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

To confirm the key role of MRGPRX2 in AD inflammation and type 2 cytokine release, an MC903-AD model was established using MrgprB2 conditional knockout (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 to those in the WT group (Figure 4a, c). Furthermore, histopathological observations showed a decrease in epidermal thickness, dermal thickness, and inflammatory infiltration in the dermis of the MrgprB2^{-/-} group than those in the WT group (Figure. 4b, 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 to 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 to that in the WT group on days 4, 7, and 14, while there were no significant differences between WT control and MrgprB2^{-/-} control mice (Figure 4f-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 mast cells 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-AD dermatitis 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 when 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 AD mice (Figure 5d-g). To further investigate the effect of the MRGPRX2/MrgprB2-tryptase axis on AD, type 2 cytokines were measured in patients with AD and AD mice. 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^{-/-} AD mice and were reduced following tryptase antagonist application in WT AD mice (Figure 5i). In summary, it

was concluded that MRGPRX2/MrgprB2 is involved in the regulation of type 2 inflammation in AD via 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, 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, d). Furthermore, IL-4, IL-13, IL-31, and TSLP levels in tissues were significantly elevated in tryptase-treated mice compared to controls. 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, e.g., tryptase, multiple chemokines, cytokines, and histamines(Metcalf et al., 1997, Wernersson and Pejler, 2014). High-affinity FcεRI on mast cells 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).

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 EASI 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, while 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 Th2 cells in AD pathogenesis has been established previously. Dysregulation of Th2 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 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 PAR-2 receptor *in vitro*(Redhu D. 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 via MRGPRX2 activation. In a study on chronic pruritus, MRGPRX2 was found to be highly expressed in pruritic AD lesions by RNA-Seq, and degranulated MCs could drive house dust mite (HDM)-induced type 2 skin inflammation by releasing SP and activating MrgprB2(Nattkemper et al., 2018, Serhan et al., 2019). However, the mechanism by 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*^{-/-} AD mice, activation of mast cells via 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 to those in untreated WT mice, indicating that tryptase stimulated non-histamine-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 at day 4, while the differences in the *MrgprB2*^{-/-} mice are more robust at day 14. This may be due to the greater role of IgE in the later stages of the MC903-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 PAR-2 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 Davender 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 due to the different structures of the mice, as 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 mast cell-specific conditional MrgprB2 knockout mice constructed by the Cre-Loxp recombinase system, which resulted in better MCs specificity and prevented any effect of other cells expressing the MrgprB2 receptor compared with MrgprB2 fully knockout mice.

MC activation via 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, although 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, Th2 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. 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 a potential new therapeutic target for treatment of AD.

MATERIALS AND METHODS

Study Approval

This study was registered at Chinese Clinical Trail Registry, and the registration number is ChiCTR2200063267. The ethical approval (2022008) was approved by Ethics Committee at The Second Affiliated Hospital of Xi'an Jiaotong University and conformed to the ethical standard. All the specimen 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.

AD patients

A single-center and case-control study was adopted. 9 case skin samples and 33 case blood samples were from Department of Dermatology, Second Affiliated Hospital of Xi'an Jiaotong University, who was diagnosed atopic dermatitis according to Williams diagnose standards without other diseases. 9 skin control samples were from Department of orthopedic, Second Affiliated Hospital of Xi'an Jiaotong University, who were diagnosed fracture patients with no infection and immune diseases. 11 blood control samples were from Physical Examination Center, Second Affiliated Hospital of Xi'an Jiaotong University.

Mice

Adult male C57BL/6 mice ageing 8 weeks were purchased from the Experimental Animal Center of Xi'an Jiaotong University. *MrgprB2* knockout mice were established based on Cre-floxp recombinase system. C57BL/6 *Cpa3-cre* mice (Stock No: 026828) were purchased from The Jackson Laboratory (USA). *MrgprB2* floxp-floxp mice were provided as a kind gift from the laboratory of Xinzhong Dong at Johns Hopkins University. *MrgprB2* conditional knockout

Cpa3-cre; MrgprB2-flox/flox (*MrgprB2^{-/-}*) mice were acquired by hybridization and backcross. The genotype was verified by quantitative real-time PCR. Six mice were used per group. C57BL/6 STOCK Kit^{W-sh}/HNihrJaeBsmJNju (Kit^{W-sh/W-sh}) mice were purchased from the Model Animal Research Centre of Nanjing University. The mice were housed in SPF animal center in Xi'an Jiaotong University with 20–25°C, a relative humidity of 40% and a day-night cycle of 12/12 h, 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) were purchased from MedChemExpress (Shanghai, China). MC903 was prepared by 100% ethanol to the concentration of 45µM. 8-Week-old male C57BL/6 mice were anesthetized with an intraperitoneal injection of 80 mg/kg 1% pentobarbital sodium. Mice were topically applied 12.5µl MC903 on both ventral and dorsal of 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), Benzamidine hydrochloride (tryptase antagonist, HY-W018781) were purchased from MedChemExpress (Shanghai, China). Tryptase was prepared by normal saline to the concentration of 10uM. Tryptase antagonist was prepared by normal saline to the concentration of 100uM. In tryptase supplementation and suppression in experiments, 20ul tryptase was applied on the ear skin of *MrgprB2^{-/-}*-AD mice for 14 consecutive days. Tryptase antagonist was applied 200ul by intragastric administration on WT-AD mice for 14 consecutive

days. As vehicle control, the same volume of normal saline was applied.

Mice inflammatory phenotype assessment

The mice inflammatory phenotype was assessed by atopic dermatitis index (ADI). ADI score was evaluated based on redness, drying, swelling and lichenification ranked 0, 1, 2, 3 and total score is the sum of these facts (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 topical application of MC903.

Scratching behavior assessment

The mice were acclimatized in an observation box for 30 minutes at 2 hours after 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 hematoxylin and eosin and were stained with toluidine blue. Hematoxylin and eosin-stained tissue was evaluated at 20× magnification. Toluidine blue staining used to label mast cells in tissues. MRGPRX2 was marked with MRGPRX2 Polyclonal Antibody by immunofluorescence staining. The degranulated status of mast cells in mice ear tissue was showed by immunofluorescence staining of avidin (primary antibody used is detailed in the Supplementary Table S1). After tissue sections 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 phosphate-buffered saline [PBS], pH 7.4) for 2 hours at 25°C, and incubated with 1:500 fluorescein isothiocyanate-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, MCs 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 the current study. And the data that support the findings of this study are available from the correspondence author Songmei Geng. Email: gengsongmei73@163.com.

CONFLICT OF INTEREST

All authors state no conflict of interest.

ACKNOWLEDGEMENT

We are grateful to Drs Xinyue Zhang, Cong Yan and Xiaona Li for their contribution in collecting blood and tissue specimens from AD patients. This work was supported by National Natural Science Foundation of China (82273526, No.82073451), Innovation Capability Support Program of Shaanxi (Program No.2022TD-48), Bethune Charitable Foundation (No.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.

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FIGURE LEGENDS

Figure 1. AD severity and type 2 cytokine release were not strongly associated with IgE level.

a. Analysis of the association between Ige levels in AD patients and their EASI scores and Pruritus VAS score (n=33). **b.** Determination of serum IL-4, IL-13 and TSLP levels in AD patients and healthy controls (HC n=11, AD n=33). **c.** Analysis of the association between Ige levels in AD patients and their serum IL-4, IL-13 and TSLP levels (n=33). **d.** Phenotypes in the ears of AD mice and the controls. **e.** Ear thickness, ADI score and scratching times in 20min of AD mice and the controls. **f.** Histopathological results of AD mice and the controls. Scale bars: 50µm. **g.** Epidermal thickness, dermal thickness, and the number of infiltrated immune cells of AD mice and the controls. **h.** Serum IgE level between MC903-treated AD mice and the controls on day4, day7 and day14. **i.** IL-4, IL-13, IL-31 and TSLP levels of ear tissue in AD mice and the controls. (n=6. Each data point represents an individual. Differences were considered significant at * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$, ns: no significant)

Figure 2. Mast cell activation and elevated MRGPRX2 expression correlate with type 2 cytokines release in AD.

a. Hematoxylin & eosin staining, toluidine blue staining, immunofluorescence staining of MRGPRX2 of skin lesions of AD patients and healthy controls. Arrows point to MCs. **b.** The number of MCs and MRGPRX2⁺ MCs in skin lesions of AD patients and healthy controls. **c.** MRGPRX2 levels in lesions of AD patients and healthy controls (n=9). **d.** Immunofluorescence staining of avidin in ear skin lesions of AD mice on day4, day7 and day14. Scale bars: 50µm,

arrows point to MCs. **e.** MRGPRX2 levels in blood samples of AD patients and healthy controls (control=11, case=33). **f-g.** The correlation of blood MRGPRX2 levels and serum IL-4, IL-13, TSLP, SP and CGRP in AD patients. (Each data point represents an individual. Differences were considered significant at ** $P < 0.01$ and *** $P < 0.001$)

Figure 3. Mast cells mediate the phenotypic regulation and type 2 cytokines release in AD.

a. Phenotypes of the ears of mast cell-deficient Kit^{W-sh/W-sh} AD mice and WT AD mice on day7. **b.** Ear thickness, ADI score and scratching times of mast cell-deficient Kit^{W-sh/W-sh} AD mice and WT AD mice (n=6). **c.** Histopathological results of mast cell-deficient Kit^{W-sh/W-sh} AD mice and WT AD mice. Scale bars: 50 μ m. **d.** Epidermal thickness, dermal thickness, and the number of infiltrated immune cells of mast cell-deficient Kit^{W-sh/W-sh} AD mice and WT AD mice (n=6). **e.** IL-4, IL-13, IL-31 and TSLP of ear tissues of mast cell-deficient Kit^{W-sh/W-sh} AD mice and WT AD mice on day7 (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 *** $P < 0.001$, ns: no significant)

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. Scale

bars: 50 μ m. **c.** The ear thickness, ADI score and scratching times in 20min of MrgprB2^{-/-} AD mice and WT AD mice. **d.** Epidermal thickness, dermal thickness, and the number of infiltrated immune cells of MrgprB2^{-/-} AD mice and WT AD mice. **e.** Serum IgE level of MrgprB2^{-/-} AD mice and WT AD mice. **f-g.** IL-4, IL-13, IL-31 and TSLP concentration in ear tissues of MrgprB2^{-/-} AD mice and WT AD mice on day0, day4, day7 and day14. (n=6. Differences were considered significant at * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$, ns: no significant)

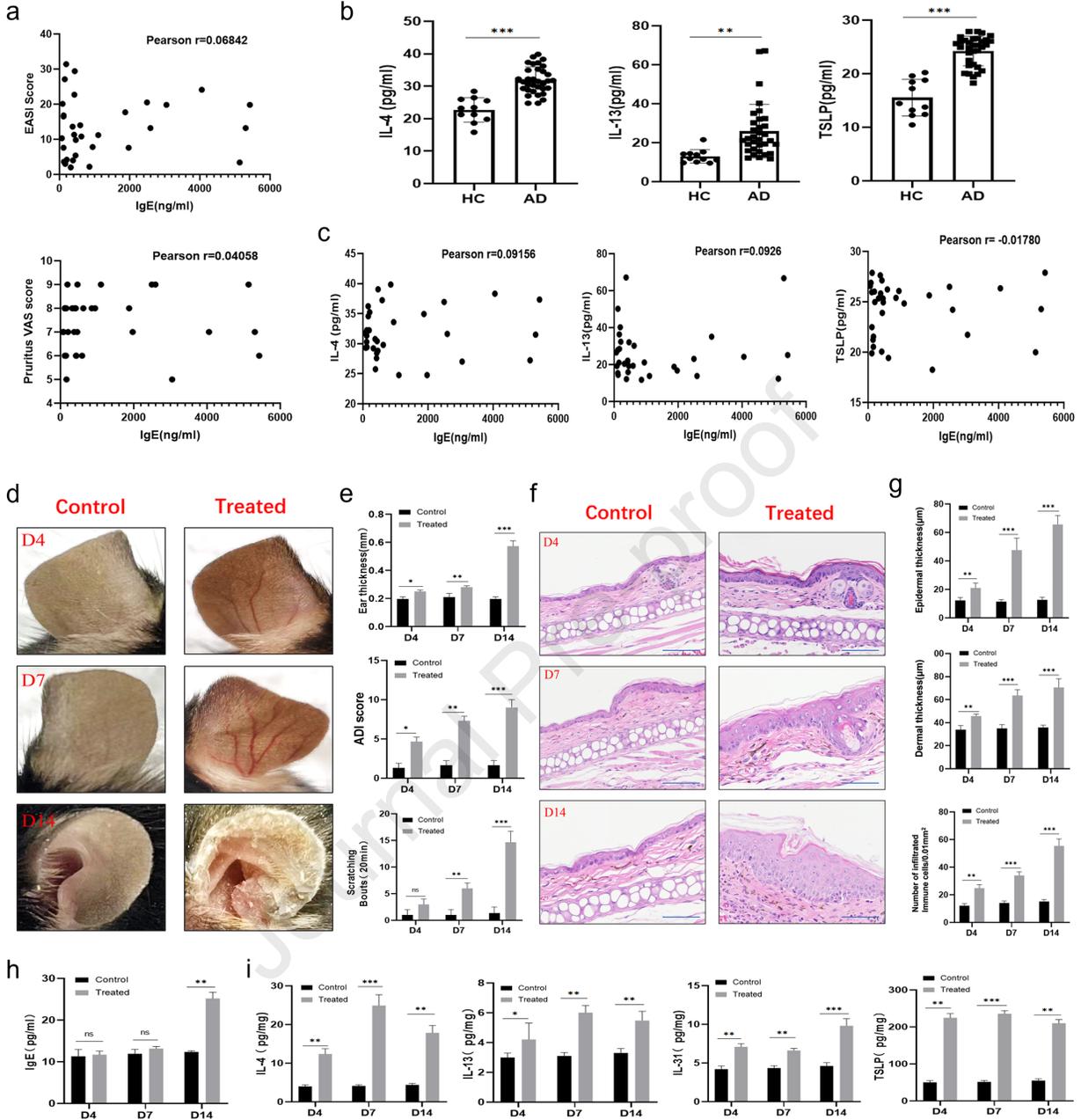
Figure 5. MRGPRX2/MrgprB2-tryptase axis contributes to AD phenotype and type 2 cytokines release.

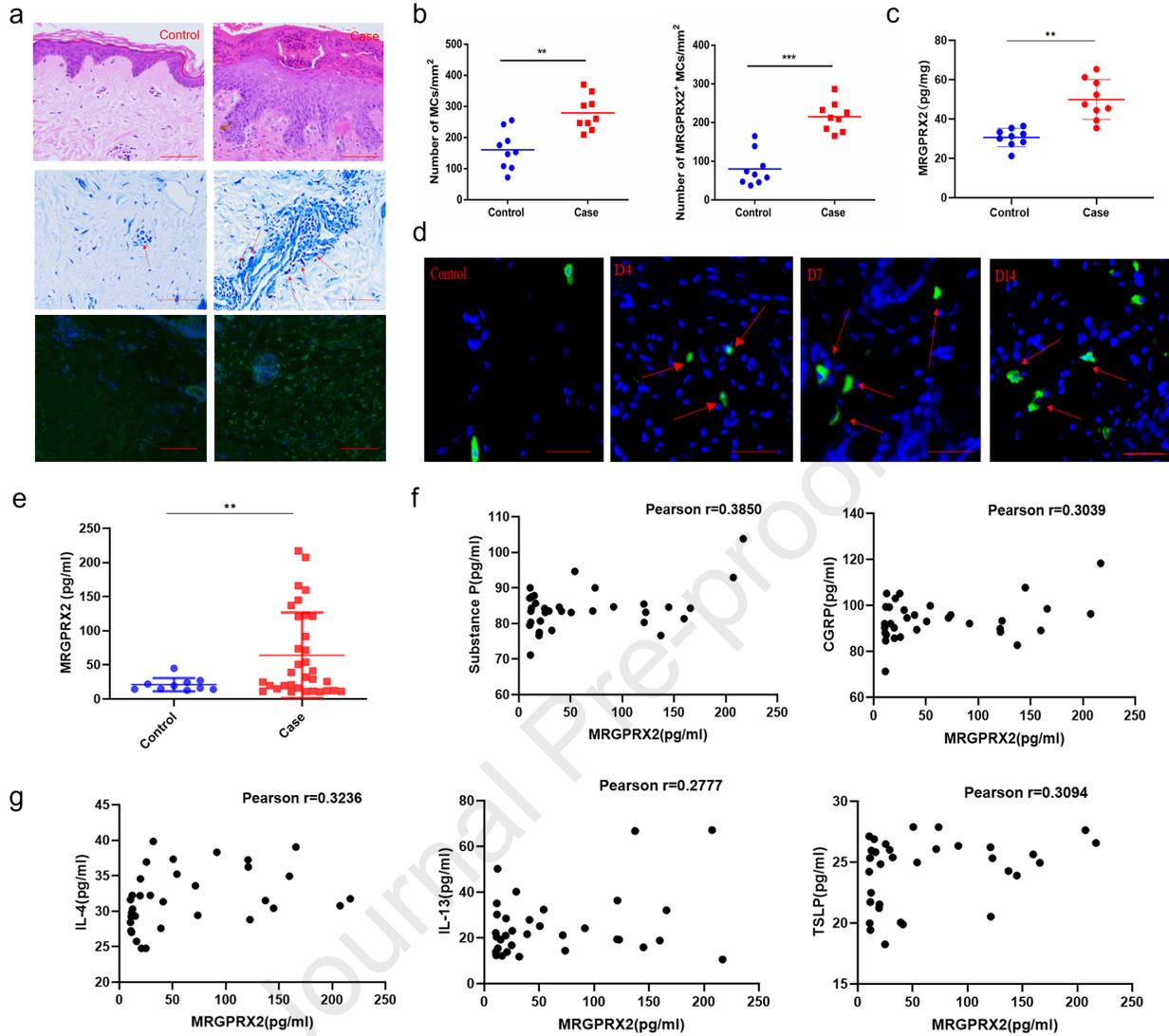
a. Serum TPS concentration of AD patients and healthy controls (control=11, case=33). **b.** The correlation between serum TPS concentration and MRGPRX2 levels in AD patients (n=33). **c.** Serum TPS concentration in the WT AD mice and MrgprB2^{-/-} AD mice (n=6). **d.** The phenotype of ears of WT-treated mice, MrgprB2^{-/-}-treated mice, MrgprB2^{-/-}-treated plus TPS application mice and WT-treated with TPS antagonist gavage mice. **e.** Ear skin histological results of WT-treated mice, MrgprB2^{-/-}-treated mice, MrgprB2^{-/-}-treated plus TPS application mice and WT-treated with TPS antagonist gavage mice. Scale bars: 50 μ m. **f.** Ear thickness, ADI score, scratching bouts in 20min of WT-treated mice, MrgprB2^{-/-}-treated mice, MrgprB2^{-/-}-treated plus TPS application mice and WT-treated with TPS antagonist gavage mice (n=6). **g.** Epidermal thickness, dermal thickness, and the number of infiltrated immune cells of WT-treated mice, MrgprB2^{-/-}-treated mice, MrgprB2^{-/-}-treated plus TPS application mice and WT-treated with TPS antagonist gavage mice (n=6). **h.** The correlation of serum IL-4, IL-13 and TSLP concentration and serum TPS concentration of AD patients (n=33). **i.** IL-4, IL-13, IL-31 and

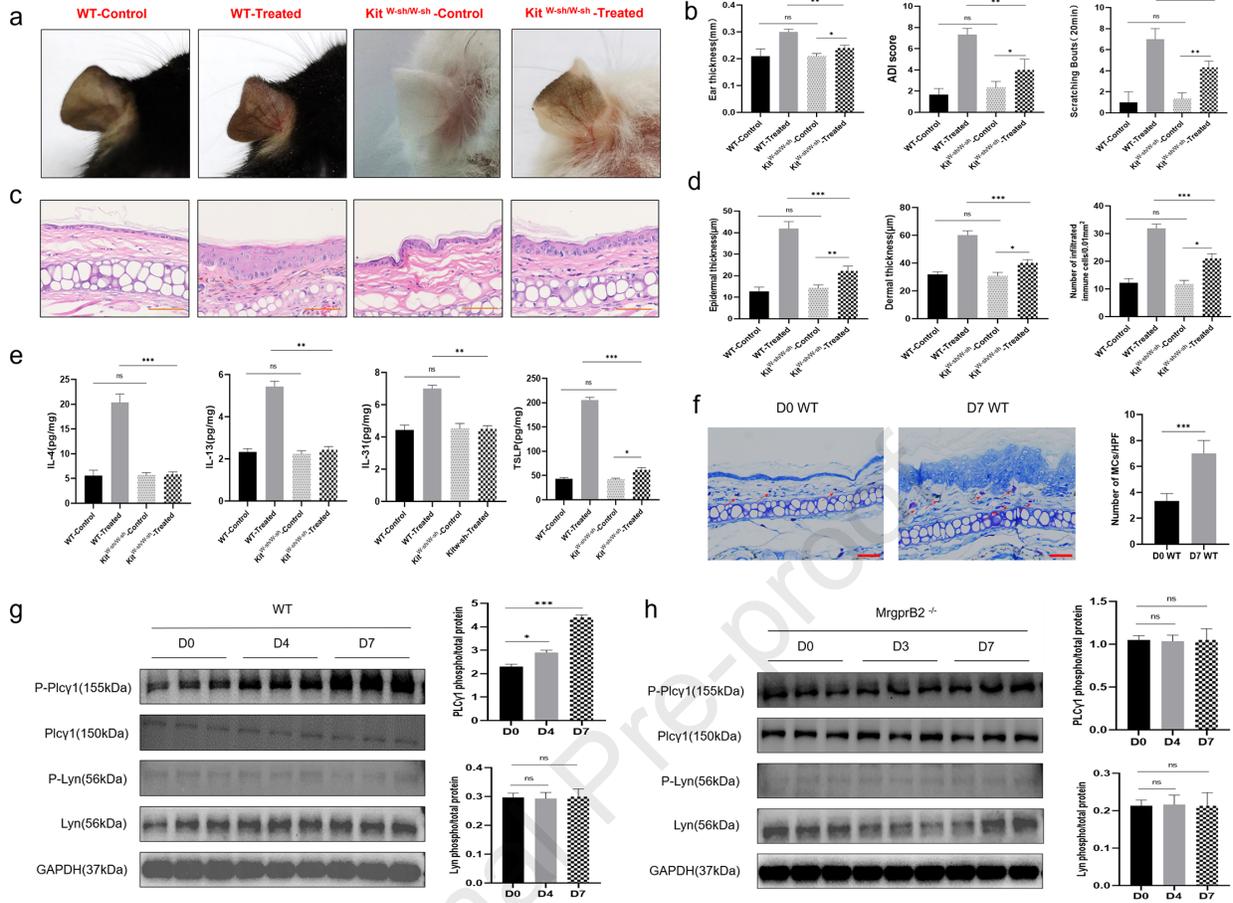
TSLP concentration in ear tissue of WT-treated mice, MrgprB2^{-/-}-treated mice, MrgprB2^{-/-}-treated plus TPS application mice and WT-treated with TPS antagonist gavage mice (n=6). (TPS, tryptase. Differences were considered significant at * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$)

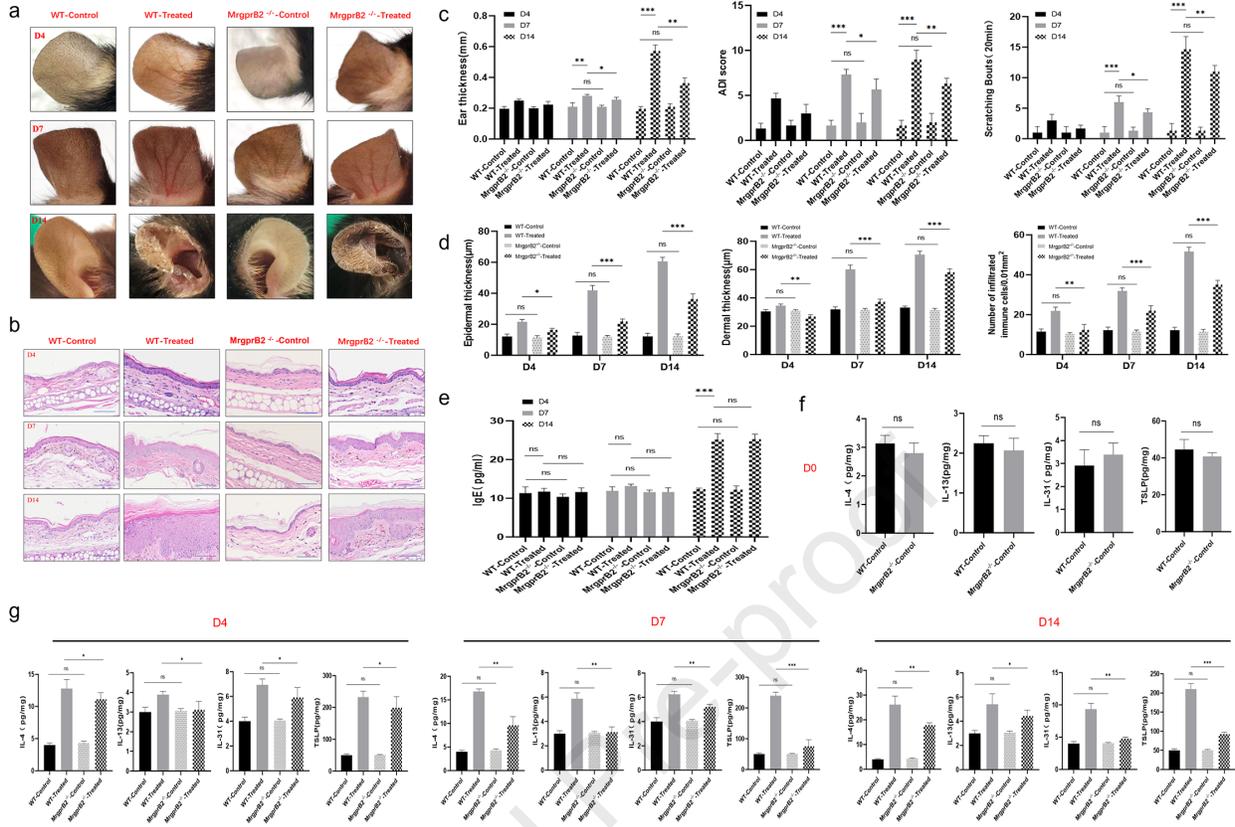
Figure 6. Tryptase could lead to type 2 inflammation *in vivo*.

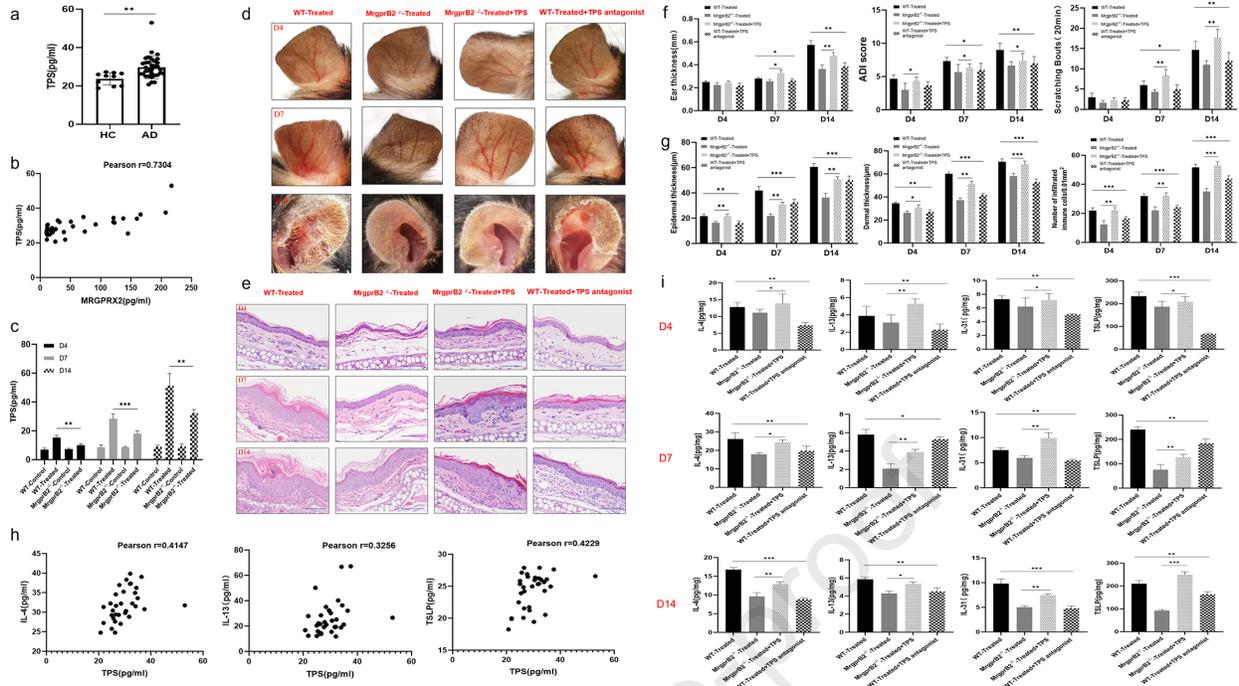
a. The phenotype of TPS-treated WT mice and saline-treated WT mice. **b.** Histopathological results of TPS-treated WT mice and the controls. Scale bars: 50 μ m. **c.** Ear thickness, ADI score and scratching bouts in 20min of TPS-treated WT mice and the controls. **d.** Epidermal thickness, dermal thickness, 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. TPS, tryptase. Differences were considered significant at * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$)

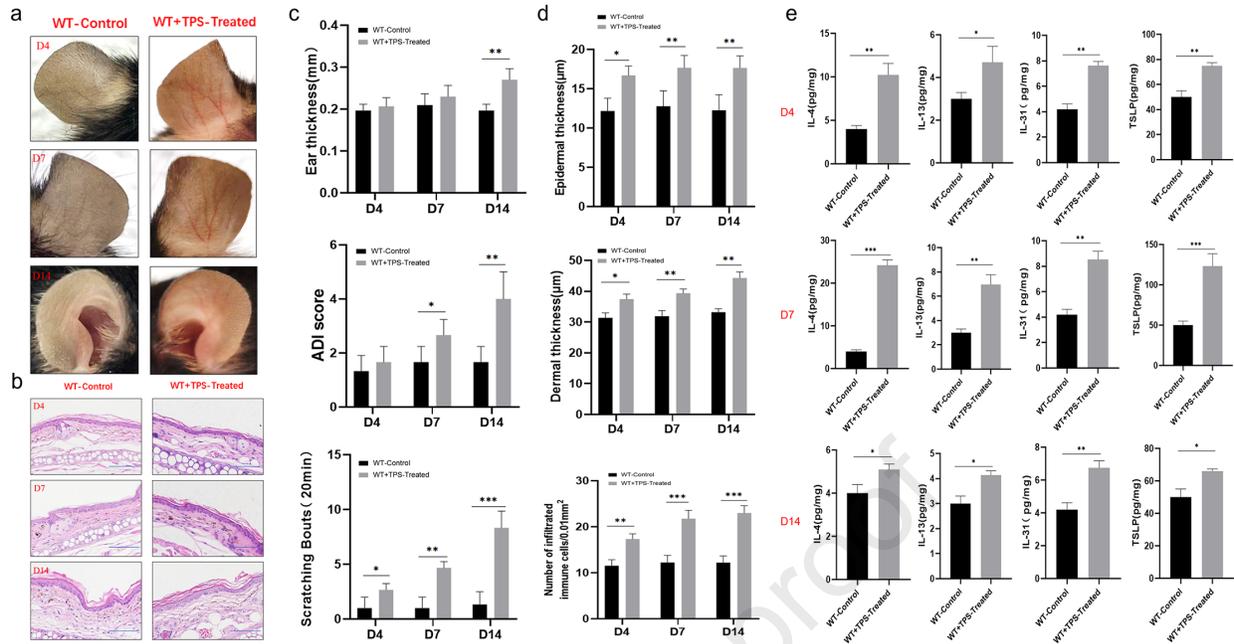






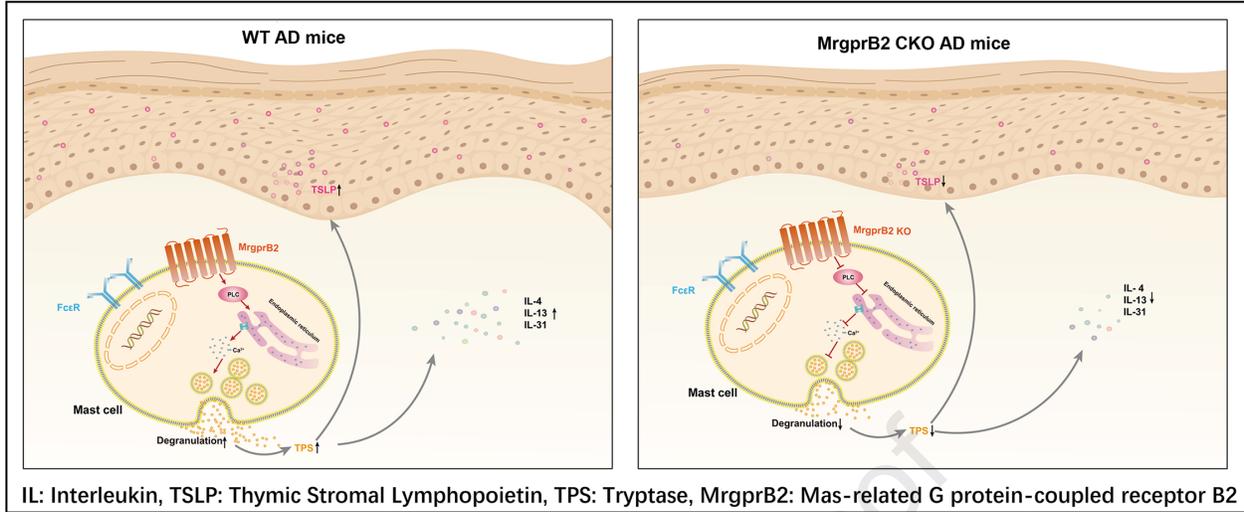






Supplementary Table S1. Antibodies used for immunohistochemistry (IHC) and western blotting (WB) analyses.

Antigen	Antibody	Species	Company	Catalog number	IHC/WB dilution
MRGPRX2	polyclonal	rabbit	Thermo	PA5-32931	1:50
Avidin	monoclonal	rabbit	Solarbio	SF065	1:150
GAPDH	polyclonal	rabbit	Proteintech	10494-1-AP	1:10000
PLC γ 1	monoclonal	rabbit	Cell Signaling	# 5690S	1:1000
p-PLC γ 1	monoclonal	rabbit	Cell Signaling	# 14008S	1:1000
Lyn	monoclonal	rabbit	Cell Signaling	# 2796S	1:1000
p-Lyn	polyclonal	rabbit	Cell Signaling	# 2731S	1:1000



SUPPLEMENTARY MATERIALS

SUPPLEMENTARY TABLES AND FIGURES

Supplementary Table S1. Antibodies used for immunohistochemistry (IHC) and western blotting (WB) analyses.

Supplementary Figure S1. Schematic representation of the involvement of MrgprB2 in the regulation of AD inflammation and type 2 cytokine release.

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SUPPLEMENTARY FIGURE LEGENDS

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 tryptase release 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.

SUPPLEMENTARY MATERIALS AND METHODS

Image acquisition, quantitative analysis

All stained sections are scanned by NDP software (HAMAMATSU NANOZOOMER DIGITAL PATHOLOGY SYSTEM) to obtain images with identical conditions of exposure and background balance. 6 independent biological replicates were chosen and measured to represent statistical significance between groups. The thickness of the epidermis and the dermis was measured using Image J software (NIH, USA). Counting of the immune cells was done manually in a defined area of the tissues. 5 random areas were selected, and its average represents the individual measurements.

ELISA

Blood and tissue samples of atopic dermatitis patients and healthy control were collected. Mice ear samples and human tissue samples were ground frozen with liquid nitrogen and added 0.5 ml saline. Then the tissues were grinded in a tissue grinder (Servicebio, Wuhan, China). The blood samples were centrifuged to obtain serum. Human MRGPRX2, tryptase, IL-4, IL-13, TSLP, SP and CGRP ELISA Kit and mouse tryptase, IL-4, IL-13, IL-31 and TSLP ELISA Kit were purchased from Shanghai MLBIO Biotechnology Co. Ltd. (Shanghai, China). And human IgE and mouse IgE ELISA Kit was purchased from Biotech (Beijing, China). Total protein content in tissues were determined using BCA Protein Assay Kit (Thermo Fisher Scientific). All steps were performed strictly according to the manufacturers' instructions.

MCs 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 150g/lane of proteins were separated by SDS-PAGE and transferred to PVDF membrane (Hangzhou Microna Membrane Technology, Hangzhou, China). Immunoblotting was done following the procedure described previously (Zheng et al., 2019), and primary antibody used is detailed in the Supplementary Table S1. Quantification of protein greyscale values was analyzed by Image J software (NIH, USA).

Data visualization and statistics

All graphical representation of data was done with 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 SPSS software. Data are expressed as mean \pm S.E.M. For comparison between two groups, Student's *t* test followed by a Mann-Whitney posttest 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: no significant.