International Immunopharmacology 81 (2020) 106258

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Imiquimod-related dermatitis is mainly mediated by mast cell degranulation via Mas-related G-protein coupled receptor B2

Yong Hao[a,b,1], Bin Peng[a,1], Delu Che[c], Yi Zheng[a], Shuzhen Kong[a], Rui Liu[c], Jihai Shi[b], Hui Han[b], Jue Wang[c], Jiao Cao[c], Yongjing Zhang[c], Jiapan Gao[c], Langchong He[c,*], Songmei Geng[a,*]

[a] Department of Dermatology, Northwest Hospital, The Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an, Shaanxi, China
[b] Department of Dermatology, The Second Affiliated Hospital of Baotou Medical College, Inner Mongolia University of Science and Technology, Baotou, Inner Mongolia, China
[c] School of Pharmacy, Xi'an Jiaotong University, Xi'an, Shaanxi, China

ARTICLE INFO

Keywords:
Imiquimod
MrgprB2
Mast cell
Contact dermatitis

ABSTRACT

While imiquimod (IMQ) has been widely used in dermatology, its side effect manifested as dermatitis couldn't be ignored. However, the underlying mechanism has not been fully understood. Considering the clinical features of IMQ-related dermatitis similar to pseudo-allergic reaction and the presence of large numbers of mast cell in tissues treated with IMQ, the possibility that IMQ-related dermatitis mediated by mast cell-specific Mas-related G protein-coupled receptor X2 (MRGPRX2) should be addressed. To investigate the role of MRGPRX2 *in vivo*, MrgprB2, the mice homology of human MRGPRX2, was detected in IMQ-induced dermatitis mouse model. Histopathological changes including mast cell degranulation and footpad swelling were assayed in wild-type and MrgprB2$^{-/-}$ mice. The results showed that IMQ application induced dermatitis and footpad swelling with inflammatory cells infiltration plus mast cell activation in the skin of wild-type mice but reduced significantly in MrgprB2$^{-/-}$ mice. Further, compared to wild-type mice, serum histamine and inflammatory cytokine levels were compromised in MrgprB2$^{-/-}$ mice treated with IMQ, while the serum IgE level didn't change significantly. *In vitro* studies, levels of mediators released from murine peritoneal mast cells (MPMCs) after IMQ treatment were increased in a dose-dependent manner, which were much mild in MPMCs from MrgprB2$^{-/-}$ mice. Intracellular Ca^{2+} concentration was increased in a dose dependent manner after IMQ treatment both in MrgprB2-HEK293 and MRGPRX2-HEK293 cells. Moreover, β-hexosaminidase released after IMQ treatment was blocked by siRNA directed at the MRGPRX2 receptor in LAD2 cells. In summary, MrgprB2 /MRGPRX2 mediate mast cell activation and participate in IMQ-related dermatitis.

1. Introduction

Imiquimod, a member of imidazoquinoline family, is a small molecule immunomodulator, which provides a topical, non-invasive treatment option for many skin diseases [1,2]. IMQ has been approved by the FDA for the treatment of external genital and perianal warts, actinic keratosis and superficial basal cell carcinoma [3,4]. In addition, it has been reported that IMQ can be used for the treatment of molluscum contagiosum [5], melanoma in situ [6], infantile hemangioma [7], verruciform xanthoma [8], cutaneous leishmaniasis [9], and porokeratosis [10]. However, the topical side effects induced by IMQ occurs frequently [11], including erythema (72.7%), pruritus (61.2%), swelling (43.8%) and pain (26.5%)[12]. The occurrence and severity of these reactions are often dose-dependent or frequency-dependent [13]. However, the mechanism of IMQ-related dermatitis isn't clear.

Pseudo-allergic reaction is a non-IgE-mediated allergic reaction with an apparent dose-dependent effect, and the most common reaction occurs in the skin, including itch, flush, angioedema and rash [14,15]. Mast cells plays a significant role in immediate hypersensitivity responses [16], and participate in various disorders and physiological processes such as drug induced anaphylactic reactions, asthma, hypertrophy, and histamine release [17]. MRGPRX2 on mast cells, which is the homology of mice MrgprB2, is the key receptor for many small molecules to induce pseudo-allergic reaction [18]. Recent studies

* Corresponding authors at: Department of Dermatology, Northwest Hospital, The Second Hospital Affiliated to Xi'an Jiaotong University, 157 Xi Wu Road, Xi'an, Shaanxi 710004, China (S. Geng). School of Pharmacy, Xi'an Jiaotong University, Xi'an, Shaanxi 710061, China (L. He).
E-mail addresses: helc@mail.xjtu.edu.cn (L. He), gsm312@yahoo.com (S. Geng).
[1] Both authors contributed equally to this work.

https://doi.org/10.1016/j.intimp.2020.106258
Received 1 October 2019; Received in revised form 20 January 2020; Accepted 24 January 2020

suggested some FDA-approved drugs, such as fluoroquinolones [19], terbinafine hydrochloride, amorolfine hydrochloride, ketoconazole, and gentamicin sulfate [20]. Caused pseudo-allergic drug reactions via MRGPRX2 in clinical practice. Intriguingly, some studies found that the number of mast cells was significantly increased in the skin lesions of patients treated with IMQ [21,22], which was also noticed in psoriasis-like dermatitis mouse model induced by IMQ [23]. Hereby, we proposed that IMQ-related dermatitis is partly a kind of pseudo-allergic drug reaction and IMQ may activate mast cell via MRGPRX2.

In this study, we investigated whether MrgprB2/MRGPRX2 played a role in IMQ-related dermatitis both *in vivo* and *in vitro*, which will help us understand the mechanism of IMQ-related side effects and provide new strategies for the prevention and treatment of IMQ-related dermatitis in clinical practice.

2. Materials and methods

2.1. Drugs and reagents

Imiquimod was purchased from Meilun Biotechnology Co., LTD (Dalian, Liaoning, China). Compound 48/80 and histamine were purchased from Sigma-Aldrich (St. Louis, USA). Histamine·2HCl (A, A, B, B-D4, 98%) was obtained from Cambridge Isotope Laboratories, Inc. (MA, USA). Mass spectrometry-grade formic acid was obtained from Sigma-Aldrich (St. Louis, USA). HPLC-grade methanol and acetonitrile were purchased from Thermo Fisher Scientific (Pittsburgh, USA). Fluo-3, AM ester, and Pluronic F-127 were obtained from Biotium (California, USA). The p-nitrophenyl N-acetyl-β-D-glucosamide, Evans blue and Triton X-100 reagents were procured from Sigma-Aldrich (St. Louis, USA). Mouse IgE, TNF-α, MCP-1, IL-23, and CXCL-2 ELISA kits were purchased from Excell Bio CO., LTD. (Beijing, China).

2.2. Mouse model

C57BL/6 male mice aged 8 weeks were purchased from the Experimental Animal Center of Xi'an Jiaotong University (Xi'an, China). C57BL/6 MrgprB2-knockout ($MrgprB2^{-/-}$) mice were provided as a kind gift from the laboratory of Xinzhong Dong at Johns Hopkins University (MD, USA). And Mast cell deficient $kit^{W-sh/W-sh}$ mice on a C57BL/6 background were purchased from the Model Animal Research Center of Nanjing University (Nanjing, China). The mice were housed in individual cages in a large colony room with 20–25 °C, with a relative humidity of 40% and a day-night cycle of 12/12 h, and free access to water and food. The mice were randomly divided into groups (6 mice per group). All animal experiments involving equal treatments were conducted by experimenters who were blinded to the conditions.

2.3. Ethics statement

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. All animals were under pentobarbital sodium anesthesia when undergoing any operation. The experimental protocols involving mice were approved by the Animal Ethics Committee at Xi'an Jiaotong University, Xi'an, China (Permit Number: XJTU 2011-0045).

2.4. Cell lines

MRGPRX2-expressing HEK293 cells were constructed by HIV-1-based lentiviral vectors, and MrgprB2-expressing HEK293 cells were provided from the laboratory of Xinzhong Dong at Johns Hopkins University. Both cells were cultured by DMEM with 10% FBS. The Laboratory of Allergic Disease 2 (LAD2) human mast cells were kindly provided by A. Kirshenbaum and D. Metcalfe (NIH, USA). The cells were cultured in StemPro-34 medium supplemented with 10 ml/L StemPro nutrient supplement, 2 mM L-glutamine, 100 ng/ml human stem cell factor (hSCF), and 1:100 penicillin-streptomycin in an atmosphere containing 5% CO_2 at 37 °C.

2.5. IMQ-induced dermatitis in mice

Eight Weeks C57BL/6 mice and MrgprB2-knockout mice were anesthetized with an intraperitoneal injection of 80 mg/kg 1% sodium pentobarbital. Then shave off the hair in the back almost 2 × 3 cm^2. Imiquimod was prepared by vaseline to the concentration of 5%. Then, 5% imiquimod 62.5 mg (approximately 40 μl) was applied on mice back daily for 3 days. Vaseline alone was set as control.

2.6. Scoring the severity of dermatitis

The dermatitis severity was assessed by using skin scoring procedure. Thus, erythema, edema, and scaling were scored independently on a scale from 0 to 4, as follows: 0, none; 1, slight; 2, moderate; 3, marked; and 4, very marked. Then, scores were accumulated (i.e., scale 0–12). The numbers of scratching bouts were counted for 20 min between 8 a.m. and 12 p.m. for 3 consecutive days. 3 of 6 mice were counted per group randomly.

2.7. Histological analysis

The skin in mice back was washed with PBS and fixed with 4% formaldehyde for 48 h and subjected to H&E staining. Stained slides were dried at 37 °C for 30 min and pre-incubated in blocking solution (10% normal goat serum (v/v), 0.2% Triton X-100 (v/v) in PBS, pH 7.4) for 2 h at 25 °C, followed by incubation with 1/500 FITC-avidin for 45 min. Sections were washed 3 times with PBS, and a drop of Fluoromount G (Southern Biotech, AL. U.S.A) was added. Images were taken using a fluorescence microscope (Nikon, Tokyo, Japan).

2.8. Analysis of mouse serum samples

Mouse IgE, TNF-α, MCP-1, IL-23 and CXCL-2 levels were determined by using ELISA kits according to provided instructions. An LC-MS 8040 mass spectrometer (Shimadzu Corporation, Kyoto, Japan) was used for the LC-ESI-MS/MS method. Histamine·2HCl was used as an interior label. Histamine was evaluated with the system by employing a HILIC column (Venusil HILIC, 2.1 mm × 150 mm, 3 μm, Agela Technologies, Tianjin, China) and an isocratic elution with acetonitrile–water containing 0.1% formic acid and 20 mM ammonium formate (77:23, v/v) at a flow rate of 0.3 ml/min.

2.9. Hind paw swelling and extravasation

Mice were anesthetized with an intraperitoneal injection of 80 mg/kg 1% sodium pentobarbital. Each mouse was injected intravenously with 0.2 ml of 0.4% Evans blue dye in saline. The thickness of the paws was measured by a cornier caliper. 5 μl of IMQ (0.5, 1, 2 μg/ml) was administered using a microinjector into the left paw, and saline only into the right paw as a blank control. 5 μl of a 30 μg/ml solution of compound 48/80 was injected as a positive control. Fifteen minutes later, the paw thickness was measured again and mice were sacrificed for analysis.

2.10. Peritoneal mast cell purification assay

Adult male C57BL/6 mice (8–10 weeks of age) were euthanized by CO_2 inhalation. A total of 12 ml of ice-cold mast cell dissociation media (MCDM; HBSS with 3% FBS and 10 mM HEPES, pH 7.2) was used to make two to three sequential peritoneal lavages, which were centrifuged at 200 × g and 4 °C for 5 min. Lavage cells were isolated by magnetic bead separation. Anti-mouse CD117 and anti-Rphycoerythrin

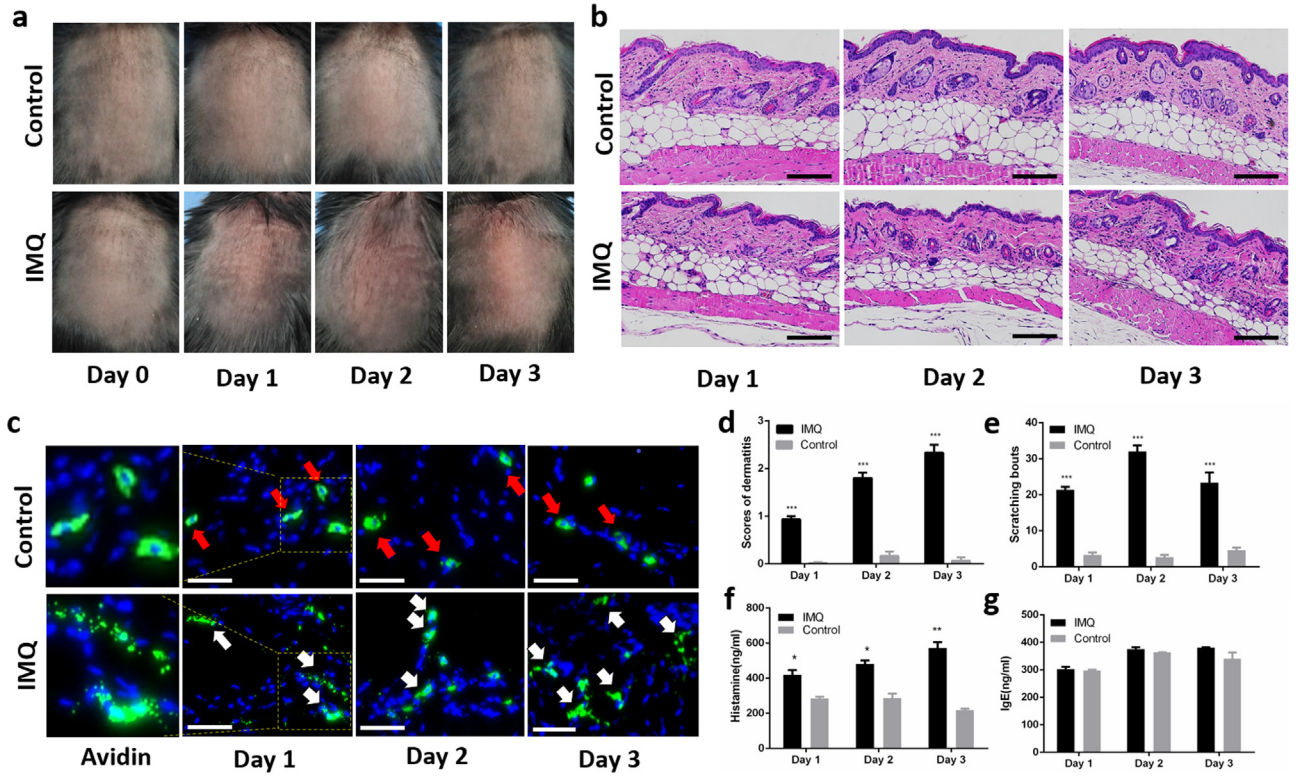

Fig. 1. IMQ induced IgE-independent mast cells degranulation in mice dermatitis. Dorsal skin treated with 5% IMQ or vaseline (Control)for 3 consecutive days are presented. **(a)** Photographs of the dorsal skin, **(b)** H&E staining of skin lesions (scale bars represent 100 μm), and **(c)** Avidin-staining of mast cells (scale bars represent 20 μm) are represented. Red arrow points to non-degranulated mast cells, white arrows point to degranulated mast cells. **(d)** Dermatitis scores were evaluated 24 h after topical treatment. **(e)** Scratching bouts were counted for 20 min daily. **(f)** Serum histamine concentration, and **(g)** IgE concentration were tested. (n = 6 mice per group). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(PE) magnetic particles (BD Biosciences, New York) were used for isolation and purification of mouse peritoneal mast cells (MPMCs). The mast cells in the pellet were retrieved, and their purity was > 95% as assayed by morphology. The mast cells were then resuspended in DMEM with 100 ng/ml recombinant mouse SCF, seeded into 96-well plates, and used within 4 h.

2.11. β-Hexosaminidase assay

LAD2 cells were incubated in a 96-well plate overnight at 37 °C with 5% CO_2. IMQ prepared by Tyrode's solution buffer to make the indicated concentrations (0.5, 1, 2 μg/ml), and the cells were incubated for 30 min. The percentage of β-hexosaminidase release was assessed by measuring the absorption of the samples at 405 nm. 30 μg/ml Compound 48/80 was used as a positive control, and Tyrode's solution buffer alone was a blank control.

2.12. Small interfering (si)RNA transfection of LAD2 cells

A SMART pool of double-stranded siRNAs targeting MRGPRX2, and nonspecific siRNAs were obtained from Shanghai GenePharma Co., Ltd. Specific knockdown was achieved using small interfering (si)RNAs targeting MRGPRX2, and non-targeting siRNAs as the negative control (NC). The siRNA sequences were as follows: forward, 5′-GUACAACAG UGAAUGGAAATT-3′ and reverse, 5′-UUUCCAUUCACUGUUGUACTT-3′ for MRGPRX2; and forward, 5′-UUCUCCGAACGUGUCACGUTT-3′ and reverse, 5′-ACGUGACACGUUCGGAGAATT-3′ for the control. For transfection, siRNA was delivered at a final concentration of 80 nM using the Lipofectamine® 2000 reagent according to the manufacturer's instructions. The cells were incubated for 48 h to allow the knockdown of MRGPRX2. These cells were then used for the β-hexosaminidase assay.

2.13. Intracellular calcium ion (Ca^{2+}) mobilization assay

Intracellular Ca^{2+} mobilization assay was performed as described previously [24]. Briefly, MRGPRX2-HEK293 cells and MrgprB2-HEK293 cells were plated in 96-well plate and incubated overnight. The cells were then washed twice with calcium imaging buffer (CIB: 125 mM NaCl, 3 mM KCl, 2.5 mM $CaCl_2$, 0.6 mM $MgCl_2$, 10 mM HEPES, 20 mM glucose, 1.2 mM $NaHCO_3$ and 20 mM sucrose, with the pH adjusted to 7.4 using NaOH). Next, 100 μl of incubation buffer was added, and the plate was incubated for 30 min and washed twice with CIB. The cells were used immediately for imaging. For the calcium imaging, the cells were magnified 200 times, and one photo per second was obtained under blue light. Cells were identified as responding if the Ca^{2+} rose by at least 50% after the substances were injected.

2.14. Statistical analysis

Experiments were repeated 3 times at least. Data were analyzed using two-tailed unpaired student's *t*-test, and presented as mean ± S.E.M. An independent samples analysis of variance was used to determine statistical significance in comparisons of the data using the SPSS 18.0 software. Differences were considered significant at *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.005$.

3. Results

3.1. IMQ induced IgE-independent mast cells degranulation in mice dermatitis

C57BL/6 mice were topically treated with IMQ on the shaved dorsal skin for 3 consecutive days. After 1 day of treatment, the dorsal skin began to show redness and scaliness, and the symptoms gradually worsened with the continued treatment. No significant skin changes were observed in the control group (Vaseline treated) (Fig. 1a). H&E staining of IMQ-treated skin showed prominent inflammatory cells infiltration in dermal compared to the control (Fig. 1b). The avidin-staining of IMQ-treated skin showed mast cells were significantly activated to undergo degranulation (Fig. 1c). Dermatitis scores of the treated and control mice showed significant difference (Fig. 1d). And the numbers of scratching were also obviously different (Fig. 1e). The IMQ application caused the increase of serum histamine level (Fig. 1f), while there was no significant difference in the IgE concentrations (Fig. 1g), which suggested that IMQ activated mast cells degranulation in an IgE-independent manner.

3.2. IMQ induced the inflammatory process of dermatitis via MrgprB2

C57BL/6 (WT) and MrgprB2-knockout (MUT) mice were treated with 5% IMQ on the shaved dorsal skin daily for 3 consecutive days. After 3 days, the serum and dorsal skin samples were collected. Compared to prominent dermatitis in WT mice, the MUT mice showed much reduced dermatitis during IMQ treatment (Fig. 2a). H&E staining of the MUT skin showed much reduced inflammatory cells infiltration in dermal compared to WT (Fig. 2b). MUT mice displayed reduced dermatitis scores (Fig. 2c) and the numbers of scratching (Fig. 2d). Level of serum histamine in MUT mice reduced significantly compared to WT mice (Fig. 2e), while there was no significant difference in IgE levels (Fig. 2f).

3.3. IMQ activated mast cells to release mediators via MrgprB2 in vivo

WT and MUT mice were treated with 5% IMQ as described above. After 3 days, with the avidin-staining showed that the mast cells in WT skin were significantly activated compared to MUT skin (Fig. 3a), the levels of MCP-1, TNF-α, CXCL-2 and IL-23 in WT mice serum also significantly increased compared to MUT mice. (Fig. 3b–e).

3.4. IMQ induced local pseudo-allergic reaction via MrgprB2

To verify whether IMQ can induce local pseudo-allergic reaction via MrgprB2, we injected IMQ into the footpad of the WT mice. The result showed that IMQ injection (left paw) evoked extensive extravasation and swelling in mice in a dose-dependent manner compared to saline injection (right paw). C48/80 injection was used as a positive control. Paw thickness increase rate and Evans blue dye exudation of IMQ injection were much higher compared to saline injection in a dose-dependent manner. (Fig. 4a). When IMQ was injected to the footpads of $kit^{W-sh/W-sh}$ and MUT mice, the swelling and extravasation were much reduced (Fig. 4b), and paw thickness increase rate and EBD exudation were much reduced in MUT mice than in WT mice (Fig. 4c). Collectively, these results indicated that IMQ induced local pseudo-allergic reaction via MrgprB2.

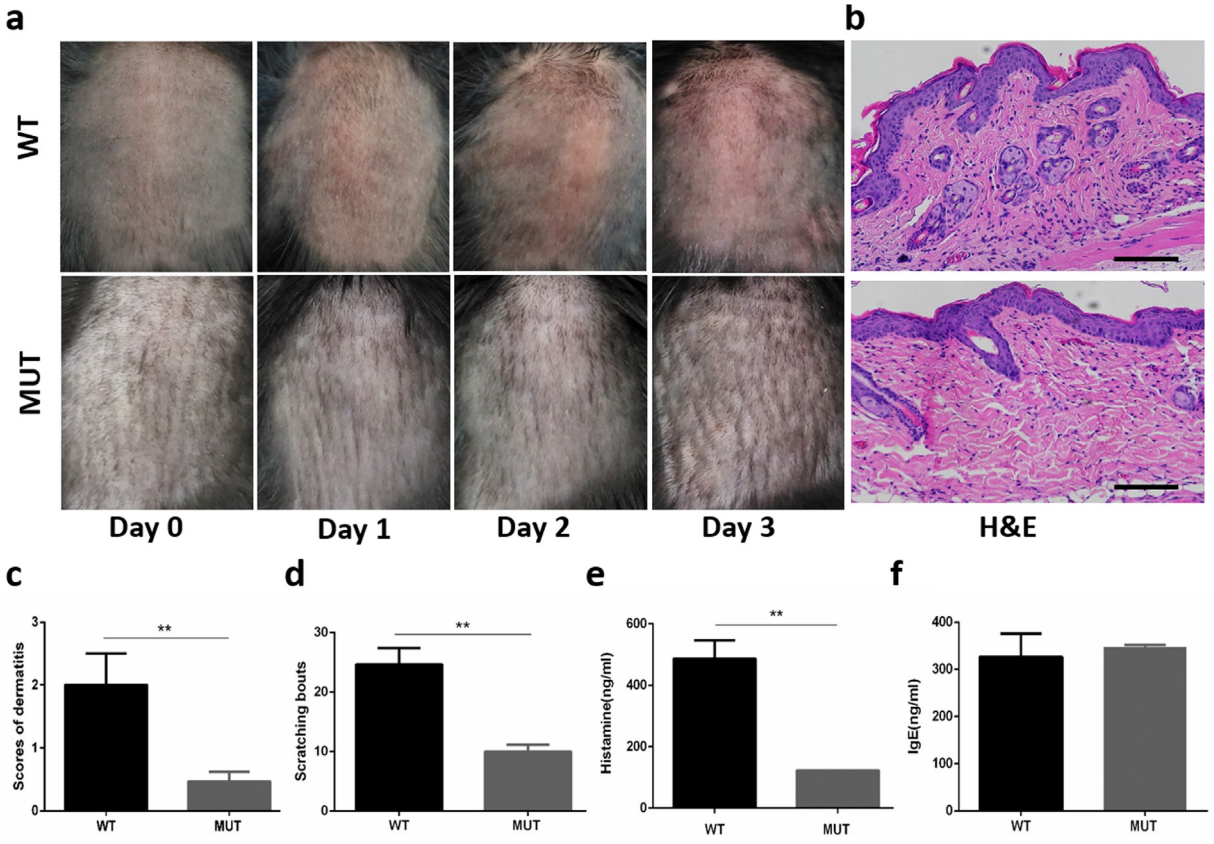

Fig. 2. IMQ induced the inflammatory process of dermatitis via MrgprB2. Dorsal skin of wild-type (WT) and MrgprB2-knockout (MUT) mice treated with 5% IMQ for 3 consecutive days are presented. **(a)** Photographs of the dorsal skin and **(b)** H&E staining in skin lesions are represented. Scale bars represent 100 μm. **(c)** Dermatitis scores were evaluated 24 h after topical treatment. **(d)** Scratching bouts were counted for 20 min daily. **(e)** Serum histamine concentration. **(f)** IgE concentration in serum. (n = 6 mice per group).

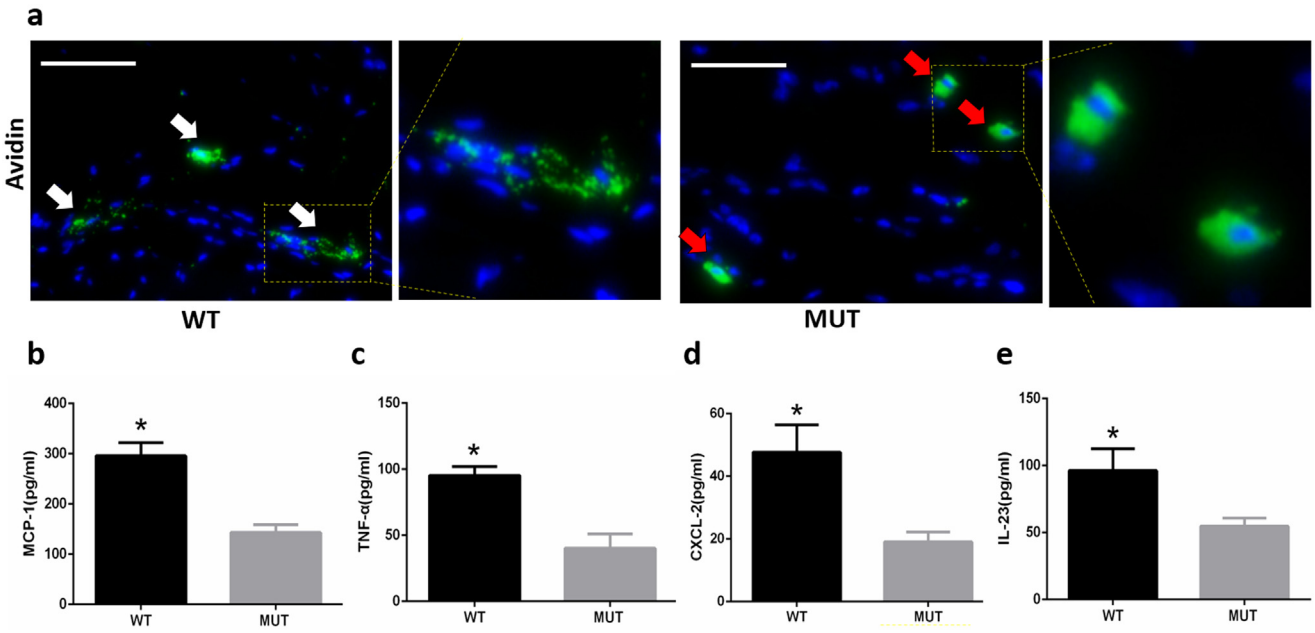

Fig. 3. IMQ activated mast cells to release mediators via MrgprB2 *in vivo*. The dorsal skin of wild-type (WT) and MrgprB2-knockout (MUT) mice treated with 5% IMQ for 3 days are presented. **(a)** Avidin-staining of mast cells in skin lesions and **(b–e)** Levels of MCP-1, TNF-α, CXCL-2, and IL-23 in serum are represented. Scale bars represent 20 μm. Red arrows point to non-degranulated mast cells, white arrow points to degranulated mast cells. (n = 6 mice per group).

3.5. IMQ induced murine peritoneal mast cells degranulation via MrgprB2 in vitro

In order to confirm whether IMQ activated mast cells via MrgprB2, MPMCs were obtained from WT and MUT mice. Varying concentrations (0.5, 1, and 2 μg/ml) of IMQ treatment to WT MPMCs showed dose-dependent increases in release of β-hexosaminidase, histamine, MCP-1 and CXCL-2. For mast cell degranulation analysis, the cells were incubated for 30 min (n = 5). And for cytokine release analysis, the cells were incubated for 8 h (n = 5). However, IMQ treatment didn't increase these mediator levels released from MUT MPMCs (Fig. 5a–d), indicating that IMQ could activate murine mast cells via MrgprB2.

3.6. IMQ induced human mast cells degranulation via MRGPRX2 in vitro

Further investigations indicated that IMQ could increase the

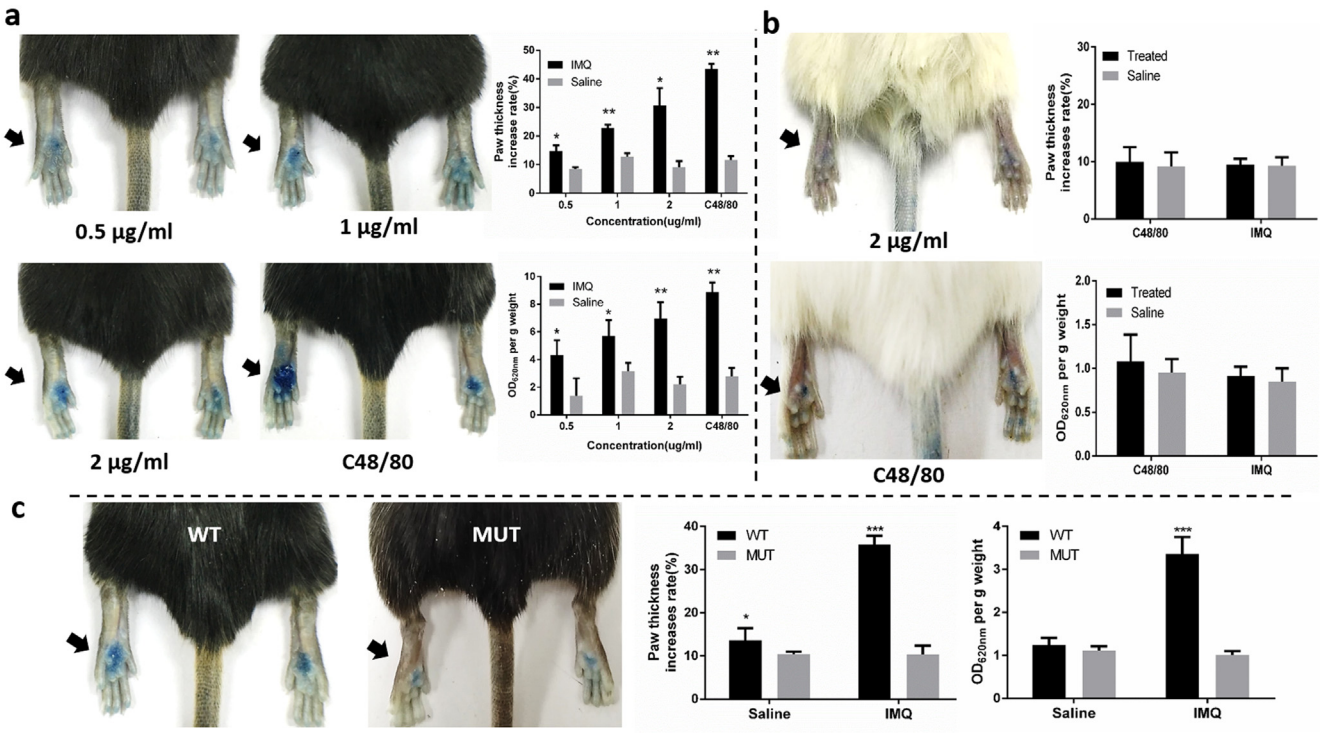

Fig. 4. IMQ induced local pseudo-allergic reaction via MrgprB2. **(a)** Representative images showing Evans blue dye extravasation in 15 min after footpad injection of IMQ (left paw), C48/80 (left paw), or saline (right paw). Quantification of paw thickness after treatment. Quantification of Evans blue leakage after treatment. **(b–c)** The same evaluation after footpad injection of IMQ in kit$^{W-sh/W-sh}$ mice, MrgprB2-knockout (MUT) and WT mice. (n = 5 mice per group).

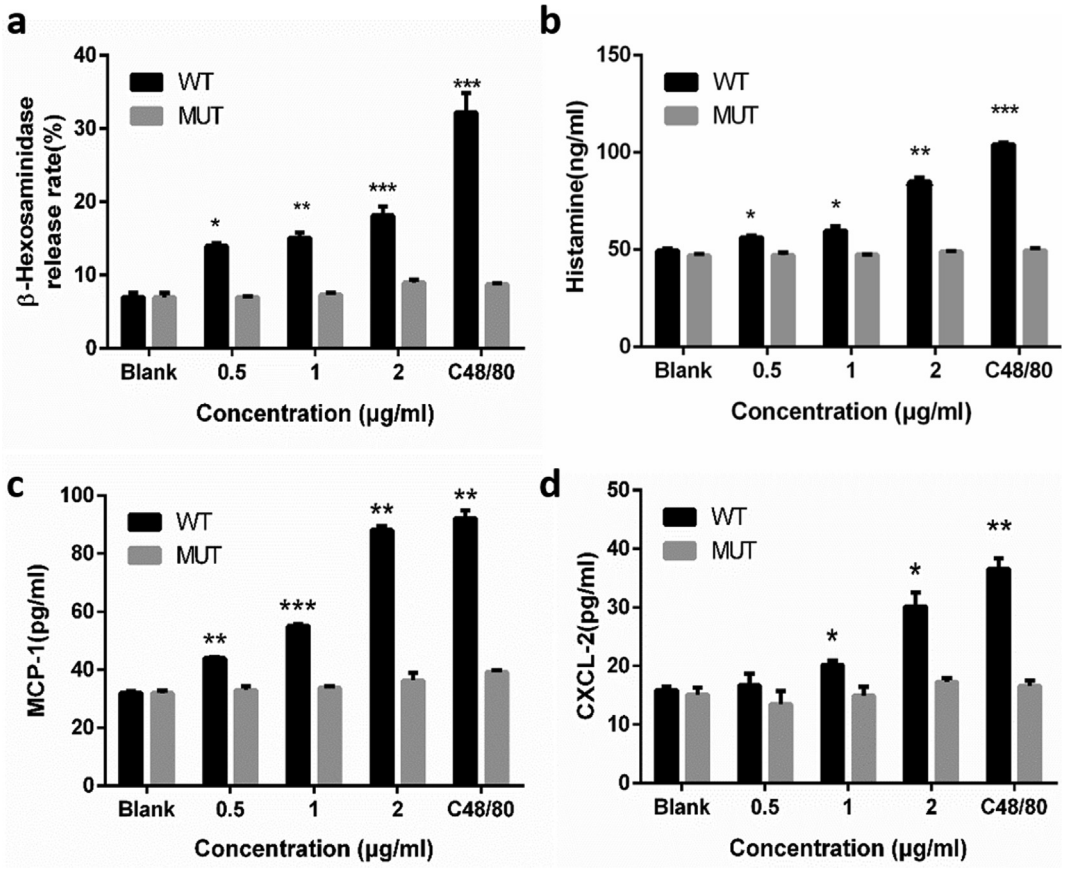

Fig. 5. IMQ induced murine peritoneal mast cells degranulation via MrgprB2 *in vitro*. MPMCs from wild-type (WT) and MrgprB2-knockout (MUT) mice were respectively treated with 0.5, 1 and 2 μg/ml IMQ. **(a–d)** The secretion of β-hexosaminidase, histamine, MCP-1 and CXCL-2 in WT MPMCs were presented. The secretion in MUT MPMCs compared with WT MPMCs.

intracellular calcium concentration in a dose-dependent manner in HEK293 cells overexpressing MrgprB2/MRGPRX2 when compared to NC-HEK293 cells (Fig. 6a–c). Interestingly, the increase in calcium concentration in MrgprB2-HEK293 cells was much greater than that in MRGPRX2-HEK293 cells at the same concentration, indicating that MrgprB2 was more sensitive to IMQ. In addition, IMQ increased the level of released β-hexosaminidase in a dose-dependent manner and the increase was prevented by siRNA directed at the MRGPRX2 receptor in the human mast cell line LAD2 cells (Fig. 6d).

4. Discussion

The most common side effect of IMQ is local dermatitis at the treatment site, and some patients experienced side effects at the first encounter with IMQ. Studies have shown that the number of mast cells increased in AK and Bowenoid papulosis lesions after IMQ treatment [21,22], which suggested that mast cells may be involved in the process of IMQ-induced immune regulation. Mast cells are critical immunocytes that function as sentinel cells in host defense reactions. It had been proven that mast cells were crucial for early inflammation, migration of Langerhans cells, and CTL (cytotoxic T lymphocyte) responses in IMQ-treated mice [25]. Our study found that the number of activated mast cells increased in IMQ-treated mice. Moreover, the activation of mast cells in IMQ-related dermatitis was depended on MrgprB2 instead of IgE, which suggested that MrgprB2 may be an important target for mast cells activation in dermatitis in mice.

MRGPRX2, a membrane receptor of mast cell, is a novel atypical opioid receptor that is mainly expressed in the human skin (MC_{TC}) [26]. Recent studies have shown that it is related to erythroderma syndrome, contact dermatitis, chronic urticaria, and atopic dermatitis [27–29]. As the orthologue of human MRGPRX2, our study detected mouse MrgprB2 and showed that IMQ could activate mast cells via MrgprB2 to induce pseudo-allergic reaction in mice.

Previous studies have suggested that IMQ, as an agonist of TLR7, mainly binds to the TLR7 of antigen-presenting cells, such as monocytes, macrophages, and dendritic cells, so as to activate the NF-κB pathway, which induced the secretion of pro-inflammatory factors IFN-α, TNF-α, IL-6, and IL-8. While IMQ activates innate immunity by these pro-inflammatory factors, it can also directly stimulate Th1 cells to produce cytokines such as IFN-γ to activate acquired immunity [2,30,31]. Additionally, independent of TLR7 and NF-κB signaling, IMQ may activate the inflammasome and keratinocytes [32]. All of these lead to an inflammatory response at the site of treatment, which is therapeutic, but also can be detrimental. Studies have shown the absence of TLR7 affected the function of mast cells in early stage of IMQ-induced dermatitis in mice [25]. In addition, it has been reported that IMQ can activate TLR7 on antigen presenting cells and even keratinocytes to release mediators, such as LL37[33], which also can activate mast cells via MRGPRX2[34]. Therefore, IMQ-related dermatitis may be mediated by both TLR7 and MrgprB2. Research had shown that TLR7-knockout have no significant effects in early IMQ-related dermatitis in mice, but the outstanding reduction of inflammation after 4 days [32]. However, the early inflammation of IMQ-related dermatitis was reduced in mast cell deficient $kit^{W-sh/W-sh}$ mice, and normalized after 5 days. And the early attenuation of inflammation can be recovered by the reconstitution of mast cells from wild-type mouse, but not $TLR^{-/-}$ mouse [25,32]. Our study found that the loss of MrgprB2 significantly weakened the early IMQ-related inflammation, accompanied by a decrease in activated mast cells. Based on this, we speculate that MrgprB2 is more important in the early stage of IMQ-related inflammatory

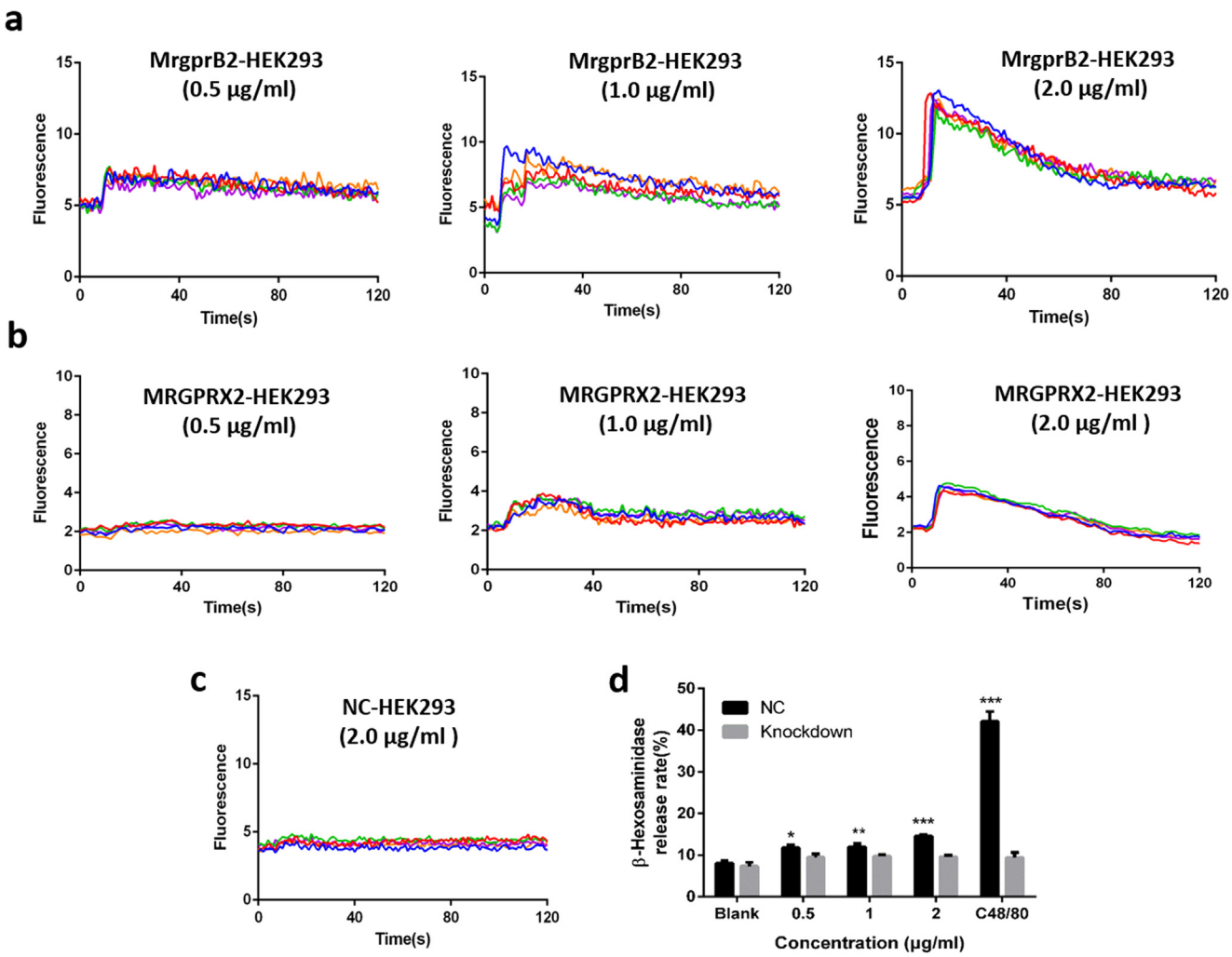

Fig. 6. IMQ induced human mast cells degranulation via MRGPRX2 *in vitro*. **(a)** MrgprB2-HEK293 cells and **(b)** MRGPRX2-HEK293 cells were treated with 0.5, 1, 2 μg/ml IMQ, representative images of Ca^{2+} concentrations are presented. **(c)** Representative images of Ca^{2+} concentrations in NC-HEK293 cells treated with 2 μg/ml IMQ. **(d)** β-Hexosaminidase release in NC-LAD2 and MRGPRX2-knockdown LAD2 cells treated with 0.5, 1, 2 μg/ml IMQ or 30 μg/ml C48/80.

response, TLR7 is effective in the later stage of inflammation, while mast cell activation and pseudo-allergic reactions via MrgprB2 may be mainly involved in early dermatitis.

Mature LAD2 cells express high levels of MRGPRX2 which is an ideal model for studying pseudo-allergic reactions [35]. And small molecule drugs could activate human LAD2 mast cells via MRGPRX2, which increases the intracellular calcium concentration and degranulation [18]. Our study showed that IMQ induced the secretion of β-hexosaminidase in a dose-dependent manner in LAD2 mast cells, and the increase was prevented by siRNA directed at the MRGPRX2 receptor, which indicated that MRGPRX2 is an important receptor for human mast cells degranulation. It has been proven TLR7 are present on the LAD2 surface, however, TLR7 activation would not lead to mast cell degranulation [36]. Therefore, we believed that IMQ activated mast cell via MrgprB2/MRGPRX2 is the main mechanism to cause pseudo-allergic reactions and eventually participates in IMQ dermatitis in mice or in human.

We found that serum levels of MCP-1, TNF-α, IL-23, and CXCL-2 were significantly increased in wild-type mice compared to MrgprB2-knockout mice, indicating MrgprB2 may play an important role in the early inflammatory response, which was consistent with other studies [37]. In addition, TNF-α and IL-23 are the major cytokines in the pathogenesis of psoriasis [38]. IMQ is frequently used to induce psoriasis-like dermatitis in mice, which is mainly characterized by erythema, thickness, and scales [39]. Considering the interaction of IMQ and MrgprB2, the IMQ-induced psoriasis-like dermatitis mouse model [40] may have more complicated pathogenesis to be investigated. Further, the presence of mast cells in human psoriasis lesions has been documented, and the early activation of cutaneous mast cells was considered as the typical finding of psoriatic inflammation [41]. Hereby, the role of MRGPRX2 on mast cells may be explored in the human psoriasis pathogenesis.

In conclusion, our study demonstrated that IMQ activated mast cells via MrgprB2 in mice, caused pseudo-allergic reaction, and eventually participated in early IMQ-related dermatitis. This may be the underlying mechanism by which IMQ causes cutaneous side effects. Our study helps to elucidate the mechanism of IMQ in regulating the immunity and provides a new possible therapeutic target for the prevention of its side effects.

CRediT authorship contribution statement

Yong Hao: Writing - original draft, Methodology, Investigation. **Bin Peng:** Methodology, Investigation. **Delu Che:** Project administration, Writing - review & editing. **Yi Zheng:** Software. **Shuzhen Kong:** Resources. **Rui Liu:** Validation. **Jihai Shi:** Resources. **Hui Han:** Data curation. **Jue Wang:** Formal analysis. **Jiao Cao:** Investigation. **Yongjing Zhang:** Resources. **Jiapan Gao:** Investigation. **Langchong**

He: Conceptualization, Resources. **Songmei Geng:** Conceptualization, Funding acquisition.

Acknowledgements

The present study was supported by the National Natural Science Foundation of China (grant No. 81171490), the Fundamental Research Funds for the Central Universities (grant No. PY3A0241001016) and the Shaanxi Provincial Key R & D Project (grant No. 2019SF-210). We would like to thank Qingyan Li, Xinyue Zhang, Qiang Zhao, Hongmei Zhou for their technical assistance and suggestions.

References

[1] E. Hanna, R. Abadi, O. Abbas, Imiquimod in dermatology: an overview, Int. J. Dermatol. 55 (2016) 831–844, https://doi.org/10.1111/ijd.13235.
[2] V.M. Voiculescu, C.V. Lisievici, M. Lupu, C. Vajaitu, C.C. Draghici, A.V. Popa, et al., Mediators of inflammation in topical therapy of skin cancers, Mediators. Inflamm. (2019) 1–15, https://doi.org/10.1155/2019/8369690.
[3] G. Micali, F. Lacarrubba, F. Dinotta, D. Massimino, M.R. Nasca, Treating skin cancer with topical cream, Expert. Opin. Pharmacother. 11 (2010) 1515–1527, https://doi.org/10.1517/14656566.2010.481284.
[4] C.A. Burns, M.D. Brown, Imiquimod for the treatment of skin cancer, Dermatol. Clin. 23 (2005) 151–164, https://doi.org/10.1016/j.det.2004.08.007.
[5] U.R. Hengge, S. Esser, T. Schultewolter, C. Behrendt, T. Meyer, E. Stockfleth, et al., Self-administered topical 5% imiquimod for the treatment of common warts and molluscum contagiosum, Brit. J. Dermatol. 143 (2000) 1026–1031, https://doi.org/10.1046/j.1365-2133.2000.03777.x.
[6] S.M. Swetter, F.W. Chen, D.D. Kim, B.M. Egbert, Imiquimod 5% cream as primary or adjuvant therapy for melanoma in situ, lentigo maligna type, J. Am. Acad. Dermatol. 72 (2015) 1047–1053, https://doi.org/10.1016/j.jaad.2015.02.008.
[7] K.R. Satterfield, C.B. Chambers, Current treatment and management of infantile hemangiomas, Surv. Ophthalmol. 64 (2019) 608–618, https://doi.org/10.1016/j.survophthal.2019.02.005.
[8] Y. Guo, Y. Dang, J.P. Toyohara, S. Geng, Successful treatment of verruciform xanthoma with imiquimod, J. Am. Acad. Dermatol. 69 (2013) e184–e186, https://doi.org/10.1016/j.jaad.2013.04.026.
[9] M.F. Peralta, H. Smith, D. Moody, S. Tristram-Nagle, D.C. Carrer, Effect of antileishmania drugs on the structural and elastic properties of ultradeformable lipid membranes, J. Phys. Chem. B 122 (2018) 7332–7339, https://doi.org/10.1021/acs.jpcb.8b04001.
[10] T. Weidner, T. Illing, D. Miguel, P. Elsner, Treatment of porokeratosis: A systematic review, Am. J. Clin. Dermatol. 18 (2017) 435–449, https://doi.org/10.1007/s40257-017-0271-3.
[11] C. Cantisani, T. Lazic, A.G. Richetta, R. Clerico, C. Mattozzi, S. Calvieri, Imiquimod 5% cream use in dermatology, side effects and recent patents, Recent Patents Inflammat. Allergy Drug Discovery 6 (2012) 65–69, https://doi.org/10.2174/187221312798889301.
[12] M.H.E. Jansen, J. Kessels, P.J. Nelemans, N. Kouloubis, A. Arits, H.P.A. van Pelt, et al., Randomized trial of four treatment approaches for actinic keratosis, New Engl. J. Med. 380 (2019) 935–946, https://doi.org/10.1056/NEJMoa1811850.
[13] H. Tran, G. Moreno, S. Shumack, Imiquimod as a dermatological therapy, Expert. Opin. Pharmacother. 5 (2004) 427–438, https://doi.org/10.1517/14656566.5.2.427.
[14] W.J. Pichler, O. Hausmann, Classification of drug hypersensitivity into allergic, p-i, and pseudo-allergic forms, Int. Arch. Allergy. Immunol. 171 (2016) 166–179, https://doi.org/10.1159/000453265.
[15] P. Kenneth, Mathews MDAA, Mich. Clinic spectrum of allergic and pseudoallergic drug teactions, J. Allergy Clin. Immunol. 74 (1984) 558.
[16] J. Singh, R. Shah, D. Singh, Targeting mast cells: uncovering prolific therapeutic role in myriad diseases, Int. Immunopharmacol. 40 (2016) 362–384, https://doi.org/10.1016/j.intimp.2016.09.019.
[17] B.M. Jensen, S. Falkencrone, P.S. Skov, Measuring histamine and cytokine release from basophils and mast cells, Methods Mol. Biol. (Clifton, NJ) 1192 (2014) 135–145, https://doi.org/10.1007/978-1-4939-1173-8_10.
[18] B.D. McNeil, P. Pundir, S. Meeker, L. Han, B.J. Undem, M. Kulka, et al., Identification of a mast-cell-specific receptor crucial for pseudo-allergic drug reactions, Nature 519 (2015) 237–241, https://doi.org/10.1038/nature14022.
[19] R. Liu, S. Hu, Y. Zhang, D. Che, J. Cao, J. Wang, et al., Mast cell-mediated hypersensitivity to fluoroquinolone is mrgprx2 dependent, Int. Immunopharm. 70 (2019) 417–427, https://doi.org/10.1016/j.intimp.2019.02.001.
[20] T. Zhang, D. Che, R. Liu, S. Han, N. Wang, Y. Zhan, et al., Typical antimicrobials induce mast cell degranulation and anaphylactoid reactions via mrgprx2 and its murine homologue mrgprb2, Eur. J. Immunol. 47 (2017) 1949–1958, https://doi.org/10.1002/eji.201746951.
[21] S. Oyama, Y. Funasaka, S.I. Tsuchiya, S. Kawana, H. Saeki, Increased number of mast cells in the dermis in actinic keratosis lesions effectively treated with imiquimod, J. Dermatol. 44 (2017) 944–949, https://doi.org/10.1111/1346-8138.13821.
[22] T. Miura, T. Yamamoto, Epidermal mast cells in bowenoid papulosis during topical imiquimod therapy, J. Dermatol. 46 (2019) e137–e138, https://doi.org/10.1111/1346-8138.14642.
[23] K.A. Cho, M. Park, Y.H. Kim, S.Y. Woo, Th17 cell-mediated immune responses promote mast cell proliferation by triggering stem cell factor in keratinocytes, Biochem. Biophys. Res. Commun. 487 (2017) 856–861, https://doi.org/10.1016/j.bbrc.2017.04.141.
[24] D. Che, L. Rui, J. Cao, J. Wang, Y. Zhang, Y. Ding, et al., Cisatracurium induces mast cell activation and pseudo-allergic reactions via mrgprx2, Int. Immunopharm. 62 (2018) 244–250, https://doi.org/10.1016/j.intimp.2018.07.020.
[25] V. Heib, M. Becker, T. Warger, G. Rechtsteiner, C. Tertilt, M. Klein, et al., Mast cells are crucial for early inflammation, migration of langerhans cells, and ctl responses following topical application of tlr7 ligand in mice, Blood 110 (2007) 946–953, https://doi.org/10.1182/blood-2006-07-036889.
[26] K. Tatemoto, Y. Nozaki, R. Tsuda, S. Konno, K. Tomura, M. Furuno, et al., Immunoglobulin e-independent activation of mast cell is mediated by mrg receptors, Biochem. Biophys. Res. Commun. 349 (2006) 1322–1328, https://doi.org/10.1016/j.bbrc.2006.08.177.
[27] D. Fujisawa, J. Kashiwakura, H. Kita, Y. Kikukawa, Y. Fujitani, T. Sasaki-Sakamoto, et al., Expression of mas-related gene x2 on mast cells is upregulated in the skin of patients with severe chronic urticaria, J. Allergy. Clin. Immunol. 134 (622–633) (2014), https://doi.org/10.1016/j.jaci.2014.05.004.
[28] E. Azimi, V.B. Reddy, E.A. Lerner, Brief communication: Mrgprx2, atopic dermatitis and red man syndrome, Itch. (Philadelphia. Pa) 2 (2017), https://doi.org/10.1097/itx.0000000000000005.
[29] Y. Zheng, D. Che, B. Peng, Y. Hao, X. Zhang, L. He, et al., All-trans-retinoic acid activated mast cells via mas-related g-protein-coupled receptor-x2 in retinoid dermatitis, Contact Dermatitis 81 (2019) 184–193, https://doi.org/10.1111/cod.13292.
[30] B. Drobits, M. Holcmann, N. Amberg, M. Swiecki, R. Grundtner, M. Hammer, et al., Imiquimod clears tumors in mice independent of adaptive immunity by converting pdcs into tumor-killing effector cells, J. Clin. Investig. 122 (2012) 575–585, https://doi.org/10.1172/jci61034.
[31] M.P. Schön, M. Schön, Imiquimod: Mode of action, Brit. J. Dermatol. 157 (Suppl 2) (2007) 8–13, https://doi.org/10.1111/j.1365-2133.2007.08265.x.
[32] B. Flutter, F.O. Nestle, Tlrs to cytokines: Mechanistic insights from the imiquimod mouse model of psoriasis, Eur. J. Immunol. 43 (2013) 3138–3146, https://doi.org/10.1002/eji.201343801.
[33] S.R. Georgescu, M. Tampa, C. Caruntu, M.I. Sarbu, C.I. Mitran, M.I. Mitran, et al., Advances in understanding the immunological pathways in psoriasis, Int. J. Mol. Sci. 20 (2019), https://doi.org/10.3390/ijms20030739.
[34] Y. Yu, Y. Zhang, Y. Zhang, Y. Lai, W. Chen, Z. Xiao, et al., Ll-37-induced human mast cell activation through g protein-coupled receptor mrgx2, Int. Immunopharm. 49 (2017) 6–12, https://doi.org/10.1016/j.intimp.2017.05.016.
[35] S.W. Kashem, H. Subramanian, S.J. Collington, P. Magotti, J.D. Lambris, H. Ali, G protein coupled receptor specificity for c3a and compound 48/80-induced degranulation in human mast cells: Roles of mas-related genes mrgx1 and mrgx2, Eur. J. Pharmacol. 668 (2011) 299–304, https://doi.org/10.1016/j.ejphar.2011.06.027.
[36] F.A. Redegeld, Y. Yu, S. Kumari, N. Charles, U. Blank, Non-ige mediated mast cell activation, Immunol. Rev. 282 (2018) 87–113, https://doi.org/10.1111/imr.12629.
[37] K. Mukai, M. Tsai, H. Saito, S.J. Galli, Mast cells as sources of cytokines, chemokines, and growth factors, Immunol. Rev. 282 (2018) 121–150, https://doi.org/10.1111/imr.12634.
[38] J. Kim, J.G. Krueger, Highly effective new treatments for psoriasis target the il-23/type 17 t cell autoimmune axis, Annu. Rev. Med. 68 (2017) 255–269, https://doi.org/10.1146/annurev-med-042915-103905.
[39] S.Y. Chuang, C.H. Lin, C.T. Sung, J.Y. Fang, Murine models of psoriasis and their usefulness for drug discovery, Expert. Opin. Drug. Discov. 13 (2018) 551–562, https://doi.org/10.1080/17460441.2018.1463214.
[40] L. van der Fits, S. Mourits, J.S. Voerman, M. Kant, L. Boon, J.D. Laman, et al., Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the il-23/il-17 axis, J. Immunol. (Baltimore, Md) 2009 (182) (1950) 5836–5845, https://doi.org/10.4049/jimmunol.0802999.
[41] L.P. Peres, F.B. Oliveira, A. Cartell, N.G. Mazzotti, T.F. Cestari, Density of mast cells and intensity of pruritus in psoriasis vulgaris: a cross sectional study, Anais brasileiros de dermatologia 93 (2018) 368–372, https://doi.org/10.1590/abd1806-4841.20186607.