Mas-related G-protein coupled receptor-B2 participates in imiquimod induced dermatitis through degranulation of mast cell

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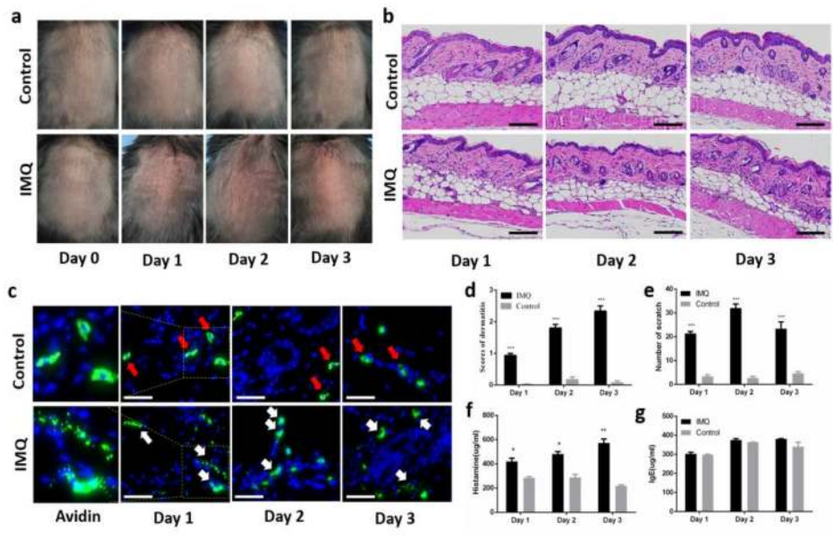

Fig. 1 Mast cells participated in IMQ dermatitis in mouse model. Dorsal skin treated with 5% IMQ and vaseline (Control)for 3 consecutive days are presented. (a) Photographs of the dorsal skin, (b) H&E staining (scale bars represent 100μm), and (c) Immunofluorescence of avidin-staining (scale bars represent 20μm). Red arrow points to non-degranulated mast cells, white arrows point to degranulated mast cells. (d) Dermatitis scores were accumulated after 24 hours topical application of drugs. (e)Scratching times in 20 minutes were counted daily. (f) Serum histamine concentration(ng/ml). (g) Serum IgE concentration(ng/ml) .

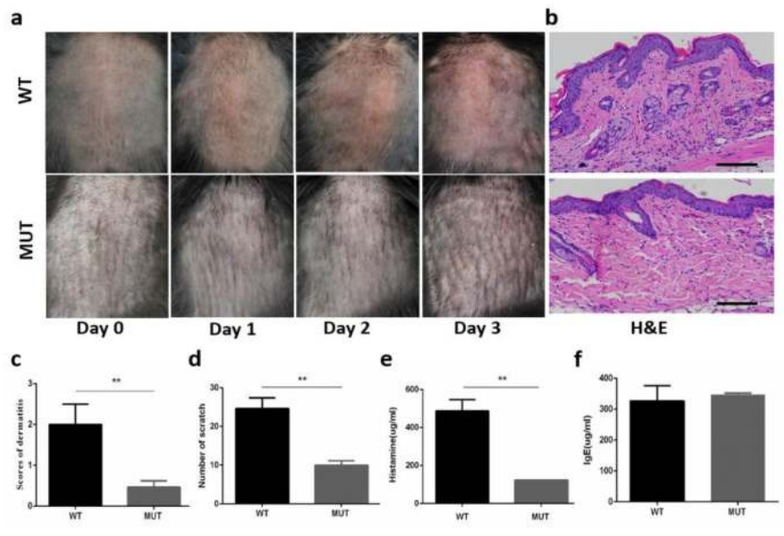

Fig. 2 MrgprB2 participated in inflammatory process in IMQ dermatitis. Dorsal skin of Wild-type (WT) and MrgprB2-knockout (MUT) treated with 5% IMQ for 3 consecutive days are presented. (a) Photographs of the dorsal skin and (b) H&E staining in skin lesions are represented. Scale bars represent 100μm. (c) Dermatitis scores were accumulated after 24 hours topical application of drugs. (d)Scratching times in 20 minutes were counted daily. (e) Serum histamine concentration(ng/mL). (f) IgE concentration(ng/ml) in serum.

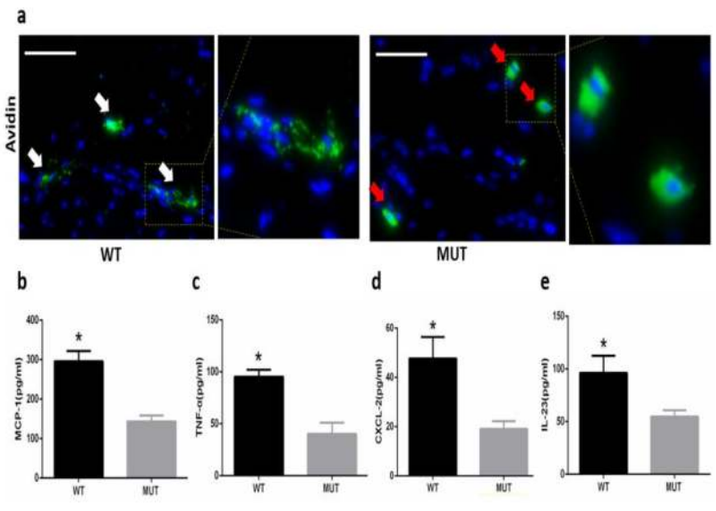

Fig. 3 IMQ induced mast cells degranulation and cytokines release via MrgprB2 *in vivo*. The dorsal skin and serum of Wild-type (WT) and MrgprB2-knockout (MUT) treated with 5% IMQ on 3 days are presented. (a) Immunofluorescence of avidin-staining in skin lesions and (b-e) Levers of MCP-1, TNF-α, CXCL-2, and IL-23 in serum are represented. Scale bars represent 20μm. Red arrows point to non-degranulated mast cells, white arrow points to degranulated mast cells.

Background: Imiquimod (IMQ) is a common drug in skin disease therapy. However, the side effects of IMQ occurred frequently, which manifested as erythema, itchy dermatitis, and the mechanism has not been fully understood. Mast cells have been found in the lesion after IMQ treatment, and MRGPRX2 on mast cells has been proved to induce pseudo-allergic reaction in chronic urticaria, contact dermatitis, and other skin diseases. Whether IMQ related dermatitis is mediated by mast cell degranulation via MrpgrB2/MRGPRX2 need to be addressed.

Objectives: To investigate whether IMQ could activate mast cell degranulation via MrgprB2/MRGPRX2 in IMQ related dermatitis.

Methods: IMQ was applied on skin of mouse for three consecutive days to establish the IMQ dermatitis mouse model. H&E staining were obtained to observe changes of histopathology while immunofluorescence was used to evaluate the degranulation of mast cells in lesions of IMQ related dermatitis. Mice hindpaw swelling, enzyme-linked immunosorbent assays, and degranulation assays were performed to measure allergic mediators both in vivo and in vitro.

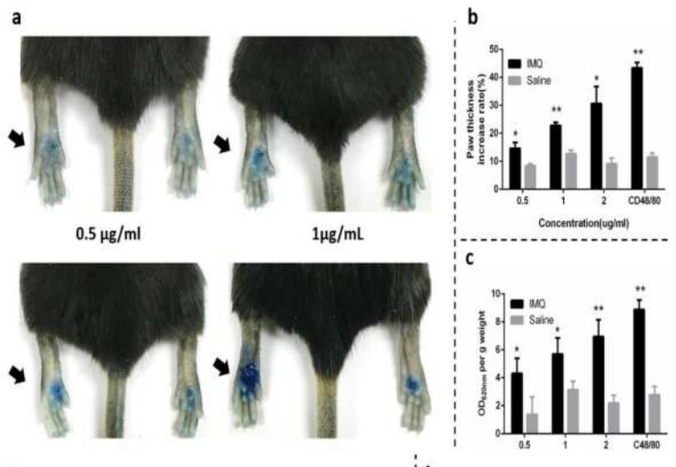

Fig. 4 IMQ induced local allergic reaction in a dose-dependent way. (a)Representative image showing Evans blue dye extravasation 15 min after intraplantar injection of 5 μl of IMQ (left paw), C48/80 (left paw), or saline (right paw). (b) Quantification of paw thickness after treatment. (c) Quantification of Evans blue leakage into the paw after treatment

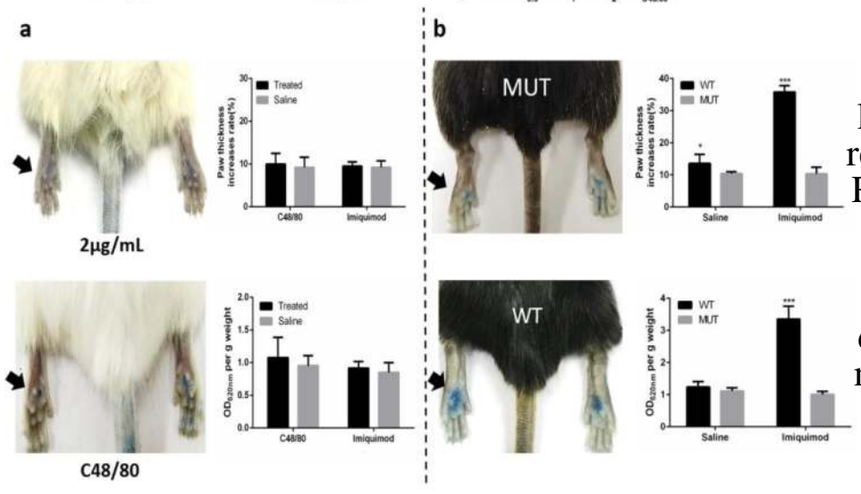

Fig. 5 IMQ induced local pseudo-allergic reaction via MrgprB2. Representative image of experiments with kit$^{W\text{-}sh/W\text{-}sh}$ mice showing Evans blue dye extravasation 15 min after intraplantar injection of 5 μl of IMQ (left paw), C48/80 (left paw), or saline (right paw). (a) Severity of local allergic reaction after treatment in kit$^{W\text{-}sh/W\text{-}sh}$ mice are presented. (b) The same evaluation after intraplantar injection of IMQ (2 μg/ml) in MrgprB2-knockout (MUT) and WT mice.

Results: IMQ induced dermatitis, paw swelling through activating mast cells degranulation and increased levels of cytokines and histamine in the serum of wild type mice, but did not increase in IgE level. In contrast, local allergic reactions were reduced or impaired in kitW-sh/W-sh and MrgprB2-knockout mice. Meanwhile, treatment with IMQ in MrgprB2-HEK293 cells showed dose-dependent increases in intracellular Ca2+ concentration, but not effectively trigger the calcium flux in NC-HEK293 cells. Further, IMQ increased the release rate of β-hexosaminidase in LAD2 cells but not in MRGPRX2 knock-down LAD2 cells.

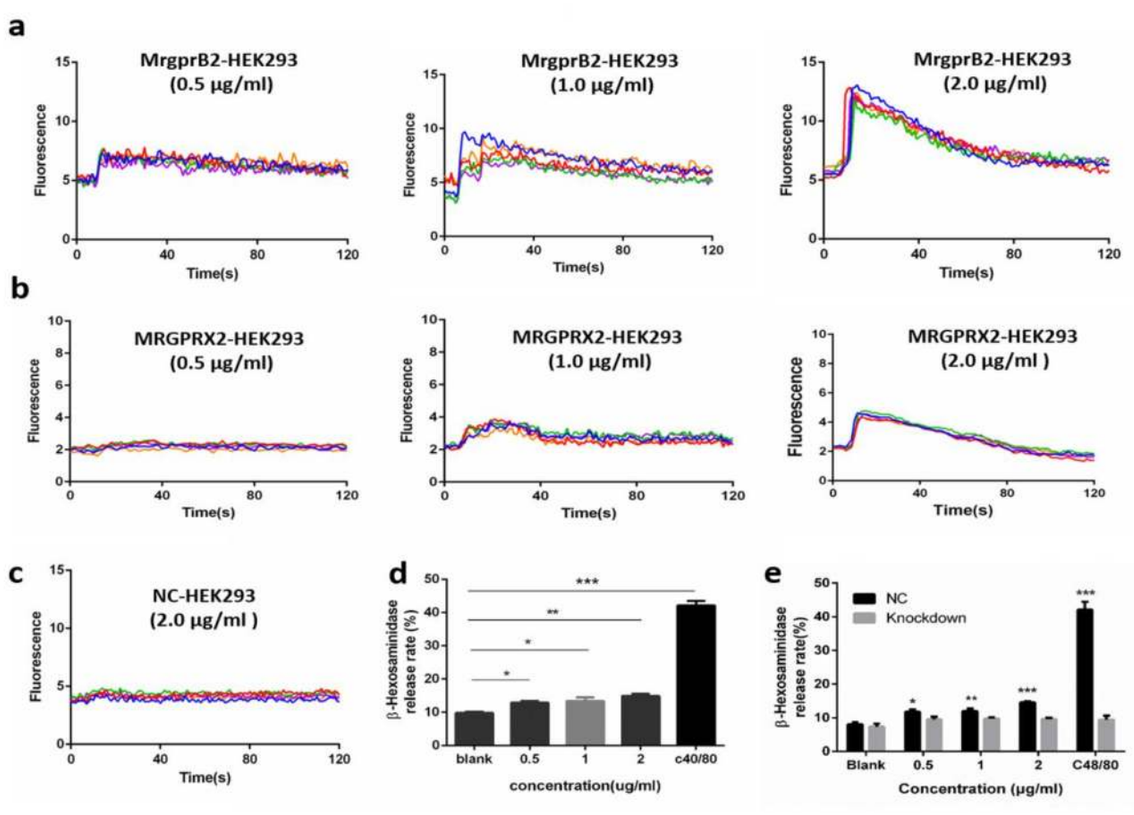

Fig. 6 IMQ induced mast cells degranulation via MRGPRX2 *in vitro*. (a) MrgprB2-HEK293 cells and (b) MrgprX2-HEK293 cells are treated with 0.5, 1, 2 μg/ml IMQ, representative images of Ca^{2+} concentrations are presented. (c)Representative images of Ca^{2+} concentrations in NC-HEK293 cells by bath application of 2 μg/ml IMQ. (d) NC transfected LAD2 cells and (e) MrgprX2 knockdown LAD2 cells are incubated with 0.5, 1, 2 μg/ml IMQ or 30 μg/ml C48/80 for 30 min, the release rate of β-hexosaminidase are presented.

Conclusions: IMQ related dermatitis may be mediated by MrgprB2/MRGPRX2 on mast cells, which lays a foundation for the possible prevention of side effects.

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