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Article type : Brief Communication

Handling AE: De Yun Wang

Brief communication

Allergic FcεRI- and pseudo-allergic MRGPRX2-triggered mast cell activation routes are independent and inversely regulated by SCF

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Short title: Allergic versus pseudo-allergic skin mast cell activation

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/all.13301

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Keywords

Mast cell, MRGPRX2, pseudo-allergy, Substance P, stem cell factor

Abbreviations

c48/80: compound 48/80

GPCR: G-protein coupled receptor

HR: histamine release

HRA: histamine release assay

MC(s): Mast cell(s)

MC_T: Mast cells expressing only tryptase

MC_{TC}: Mast cells expressing tryptase and chymase

MRGPRX2: Mas-related G-protein coupled receptor member X2

NK1: neurokinin-1

PAG-CM: **P**iperazine-N,N-bis[2-ethanesulfonic acid]-**A**lbumin-**G**lucose buffer containing 3 mM **C**aCl₂ and 1.5 mM **M**gCl₂, pH 7.4

SCF: stem cell factor

SP: Substance P

Abstract

While allergic mast cell (MC) degranulation occurs by FcεRI-aggregation and varies in strength among subjects, the analogous pseudo-allergic route was recently uncovered to proceed via MRGPRX2. Here, we examine inter-individual variability in skin MC responses to FcεRI-triggering versus those evoked by MRGPRX2. While population-based variability is comparable between the routes, FcεRI- and MRGPRX2-stimulated pathways are completely independent from each other, and responsiveness to one has therefore no predictive value for the other. Conversely, degranulation triggered by compound 48/80 is highly correlated to the process elicited by Substance P. MRGPRX2 mRNA shows pronounced population-based variability (coefficient of variation 102.9%). Surprisingly, SCF as the MC-supportive mediator *par excellence* potently inhibits pseudo-allergic degranulation, while it simultaneously promotes allergic stimulation via FcεRI. We conclude that SCF can have selective MC-dampening functions. Clinically, the data imply that subjects highly reactive in one pathway are not automatically hyper-responsive in terms of the alternative route.

Introduction

Acute mast cell (MC) activation results in a variety of symptoms and is the root of hypersensitivity disorders like (allergic) rhinoconjunctivitis, asthma, urticaria, food allergy and anaphylaxis. Symptoms are caused by mediators rapidly secreted from MCs in a process termed degranulation (1). In many instances, activation proceeds through the allergic route, which comprises three components: the high affinity IgE receptor (FcεRI) on the MC surface, IgE directed against a specific allergen bound to FcεRI, and the allergen itself which aggregates two or more FcεRI entities to ignite a cascade which culminates in exocytosis (1-3).

There are clinical reactions that resemble allergies, but do not involve immunological MC degranulation, yet do depend on degranulating MCs, and are therefore dubbed pseudo-allergic or allergoid/anaphylactoid. They can be triggered in all subjects, i.e. also in non-atopic individuals. After decades of uncertainty as to the responsible receptor, the clinically relevant “second route” was finally uncovered to proceed via the G protein-coupled receptor (GPCR) MRGPRX2 (or murine Mrgprb2) (4,5).

Ligands encompass various drugs, antimicrobial peptides, and neuropeptides like Substance P (SP), making the receptor quite universal (4-7). These ligands stimulate MRGPRX2 directly without the necessity of an intermediary like IgE. MRGPRX2 expression is confined to MCs of the MC_{TC} type, but is absent from MC_T-type cells (6-8), and skin MCs were the only constituents in our FANTOM5 body-wide atlas to express MRGPRX2 (9,10). FcεRI-mediated MC activation has been investigated for decades (1-3). We recently reported on substantial variability in FcεRI-triggered degranulation in the human population (11,12). As research into MRGPRX2 began only recently, little is known about donor dependence or its modulation by MC growth factors. Here, we therefore studied variation in MRGPRX2-triggered secretion in the population, investigated the relationship between FcεRI- and MRGPRX2-elicited responses and their modulation by stem cell factor (SCF).

Methods

Skin samples

This was an unlinked anonymous study. Donor skins, which otherwise would be disposed of, were obtained from circumcisions (foreskin), with informed consent of the patients or their legal guardians, as in previous studies from our group (9-15). No additional materials, including blood, were removed, and no skin prick tests performed. The study was approved

by the Ethics Committee of the Charité Universitätsmedizin Berlin and experiments were conducted according to the Declaration of Helsinki Principles.

Skin MC purification

MCs were isolated from skin samples, as described (11-15). Briefly, skin was cut into strips and treated with dispase (BD Biosciences, Heidelberg, Germany) at 3.5 U/ml and 4°C overnight. After removal of the epidermis, the dermis was chopped and digested with collagenase at 10 mg/ml (Worthington, Lakewood, NJ, USA) and hyaluronidase at 5 mg/ml (Sigma, Deisenhofen, Germany) for 1 h at 37°C. The cells were separated from remaining tissue by filtration. MC purification was achieved by positive selection with anti-human c-Kit microbeads and the Auto-MACS (both from Miltenyi Biotec, Bergisch Gladbach, Germany). MC purity always exceeded 98%, as assessed by acidic toluidine-blue staining (11,12,16).

Histamine release assay (HRA)

HRA was performed according to a method routinely employed in our laboratory (10-16). In brief, MCs were stimulated by FcεRI-aggregation (anti-FcεRIα-Ab 29C6 at 0.5 µg/ml), c48/80 (at 10 µg/ml), SP (at 30 µM), c48/80+SP or no stimulus (spontaneous) for 30 min at 37°C. Priming with SCF (10 ng/ml) was for 30 min before stimulation. Histamine in the supernatants was measured by an automated fluorescence method (Borgwaldt Technik, Germany, Hamburg). Total cellular histamine content was measured analogously. All determinations were performed in triplicate and histamine concentrations calculated with reference to a standard curve. Net histamine release (%) was calculated as [(stimulated release–spontaneous release)/complete histamine in the MC preparation]x100.

RT-qPCR

RT-qPCR was performed exactly as described (16).

Statistical analysis

Statistical analyses were performed with PRISM 7.0 (GraphPad Software, La Jolla, CA, USA) using Kruskal Wallis test with Dunn's multiple comparisons test to compare stimulations. Pairwise comparisons (SCF versus control) were conducted using Student's t-test. Correlations were analyzed by Spearman's correlation test. $p < 0.05$ was considered statistically significant.

Results

FcεRI- and MRGPRX2-elicited MC degranulation pathways are unconnected

We asked whether stimulability by the pseudo-allergic pathway would vary across subjects similarly to FcεRI-aggregation (11,12). By applying two ligands, namely c48/80 (extrinsic) and SP (intrinsic) (4-8) to MCs retrieved from a number of donors, we found comparable variability in MRGPRX2-triggered degranulation (Fig. 1A), as evidenced by similar CV values, i.e. 50.8, 52.5 and 58.7% for FcεRI-aggregation, c48/80 and SP, respectively.

Analyzing the datasets for correlations, we surprisingly learnt that secretion triggered by FcεRI was entirely unrelated to the one by MRGPRX2. This was true for both c48/80 (Fig. 1B) and SP (Fig. 1C). Conversely, degranulation responses were impressively linked between c48/80 and SP (Fig. 1D). Combining c48/80 with SP did not increase degranulation by either substance alone (Suppl. Fig. E1). We investigated MCs from 36 subjects for variability in MRGPRX2 mRNA expression (Suppl. Fig. E2). Variability was pronounced with

a CV of 102.9%. FcεRIα, used as control, gave a CV of 61.3%, in accordance with our previous report for a distinct cohort (11).

SCF supports allergic but inhibits pseudo-allergic degranulation of skin MCs

MC biology is dominated by the growth factor SCF, which influences nearly all aspects of the lineage in a predominantly positive fashion (2,17). In fact, in addition to growth and survival SCF also promotes degranulation via FcεRI when given acutely (2,17). Here, we simultaneously assessed its impact on the allergic and pseudo-allergic route. Short pre-incubation with the growth factor boosted histamine release triggered by FcεRI-aggregation in human skin MCs (Fig. 2A). In stark contrast, degranulation induced by either c48/80 or SP experienced a notable drop (Fig. 2B+C). When quantifying the change for the two MRGPRX2 ligands separately, we found that responsiveness to c48/80 decreased to an average of 61.6±9.4% of control (mean±SD of all individual pairs), while stimulation by SP was reduced to 73.8±15.0%. The difference in SCF-mediated change between the ligands was statistically significant (p=0.0378). Conversely, FcεRI-mediated release rose to an average of 185.3 (±76.2)% in parallel experiments.

Discussion

Understanding the activation of MCs by different classes of extrinsic challenges and their regulation by the tissue microenvironment is crucial as MC mediators are responsible for the clinical manifestations of manifold events, including life-threatening anaphylactic/anaphylactoid reactions. Differences in MC responsiveness may be an independent contributor to the manifestation of allergy-related diseases independently of sensitization (11,12,18). This study was designed to discern the relationship between the allergic and the recently uncovered pseudo-allergic route of degranulation in MCs from a

multitude of individuals and their modulation by the major MC growth factor SCF. One finding was that MRGPRX2-triggered release, much like its FcεRI-evoked counterpart, is subject to inter-individual variability, but that the two pathways are regulated independently. The clinical significance is that subjects with hyperactivity in one pathway are not automatically predisposed to react excessively regarding the alternative route.

The correlation between the two MRGPRX2 ligands SP and c48/80 (4-8) corroborates similarities in signal propagation, and different signal strength across individuals may involve variable MRGPRX2 expression (in accordance with Fig. E2) and/or functionality, as recent studies suggest that MRGPRX2 is the most relevant SP receptor on MCs (4-8). However, SP can also activate NK1, so that MRGPRX2 is not the only receptor responsive to SP in MCs (15,19). The slight difference between SP- and c48/80-evoked degranulation in the presence of SCF possibly reflects the existence of these two signaling units, whereas c48/80 seems to activate MRGPRX2 alone (4-8). Either compound triggers the maximum release achievable via the GPCR-route, indicating that the prerequisites are likely the same (receptor entity, other), and that there is an upper limit attainable via this pathway (Fig. E1)

Because SCF reportedly enhances acute stimulability by FcεRI, it came as a surprise that the pseudo-allergic route was actively suppressed by SCF. We conclude that SCF, despite its manifold lineage-supportive actions, can also have MC-dampening effects (17), and that overabundance of SCF in the tissue may even reduce the propensity to develop pseudo-allergic reactions.

Our findings harmonize with a recent report of spatially and temporally distinct granule exteriorization patterns elicited by FcεRI-aggregation vis-à-vis SP (20). While FcεRI-triggered degranulation was delayed and showed a compound exocytosis pattern, MRGPRX2-mediated degranulation was rapid and associated with a quick and transient peak of intracellular calcium followed by secretion of individual granules (20).

Together with recent evidence demonstrating that Vitamin-A metabolites favor the allergic route but counter the pseudo-allergic pathway in a chronic setting (16), the present study demonstrates that FcεRI and MRGPRX2 function can be inversely regulated by SCF in an acute scenario, suggesting that the signaling requirements differ profoundly between the routes (20). Accordingly, own preliminary findings suggest that intracellular Ca²⁺ fluxes are positively affected by SCF when triggered via FcεRI, but negatively when evoked by c48/80 (unpublished). Exploitation of the SCF counterbalance uncovered here can actually serve as a viable strategy to explore the molecular underpinnings of this “second route”

Conclusion

The clinically meaningful MC degranulation pathways are independent from each other, and inversely regulated by SCF, implying that the signaling requirements underlying a successful degranulation event are divergent between the pathways.

Figures and Legends

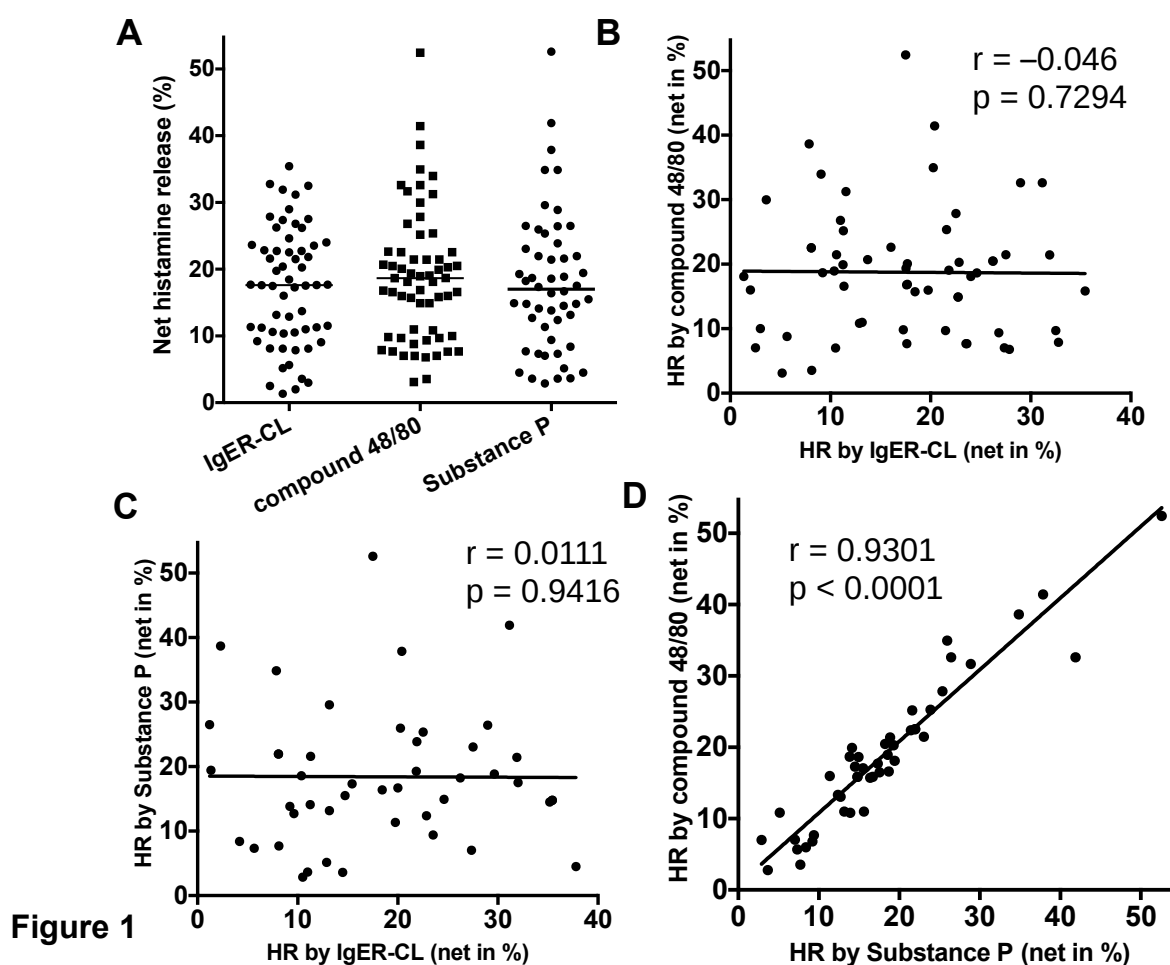


Figure 1: Despite similar variability in MRGPRX2- and FcεRI-elicited histamine release, the two routes are entirely uncorrelated in the human population

A) Activation by FcεRI-aggregation (29C6, 0.5 μg/ml; n=59), and the MRGPRX2 agonists compound 48/80 (10 μg/ml, n=61) and Substance P (30 μM, n=52) in a large number of MC preparations. B)-D) Spearman correlation analysis. B) FcεRI-aggregation vs. c48/80 (n=59), C) FcεRI-aggregation vs. SP (n=46), D) c48/80 vs. SP (n=46). No statistical difference across the groups in A (Kruskal Wallis test with Dunn's multiple comparisons test). In B-D, Spearman correlation r and associated p -values are given in the figures. HR = histamine release

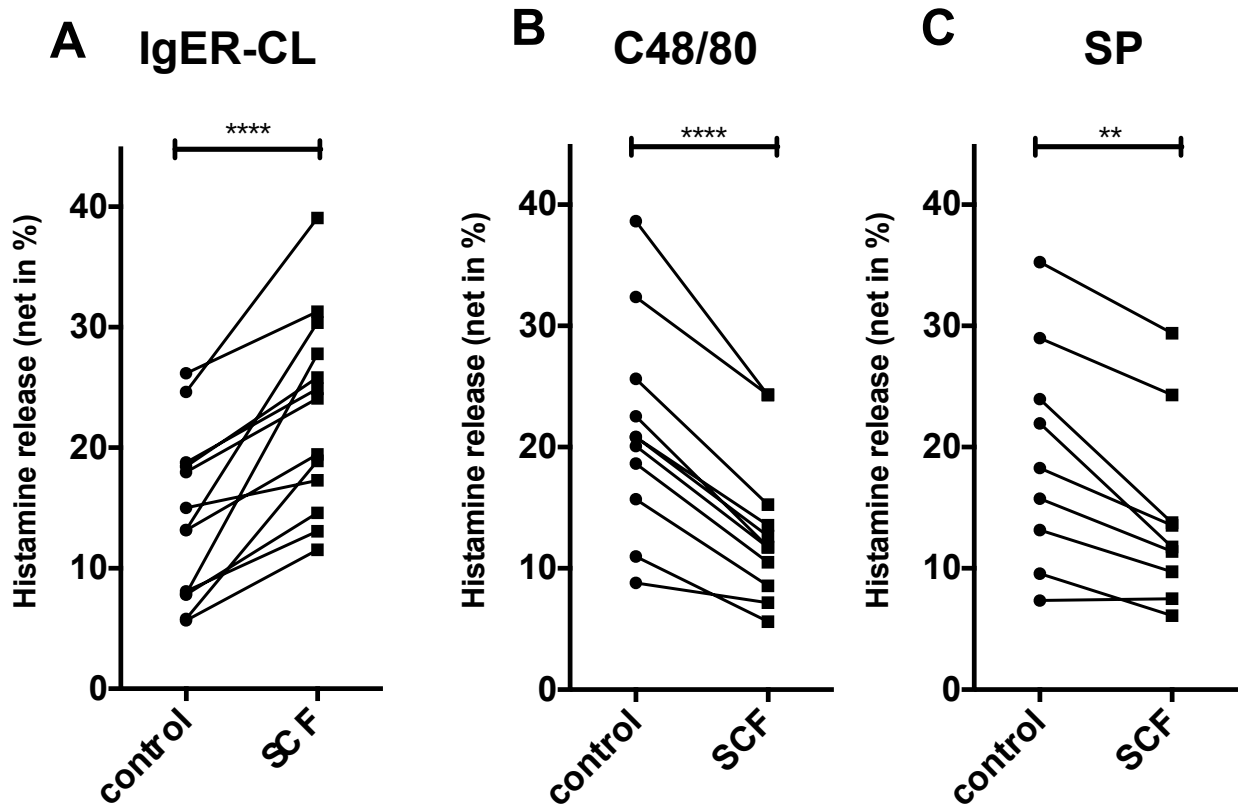


Figure 2: Priming by SCF dampens MRGPRX2-mediated, but promotes FcεRI-triggered degranulation in human skin MCs

Histamine release was assessed in paired samples of MCs exposed to SCF (10 ng/ml) or vehicle (control) for 30 min prior to stimulation. The values correspond to the net release in % (i.e. after subtraction of the corresponding spontaneous release). Histamine release by SCF alone (over the non-SCF control) was negligible ($\approx 1\%$). A) Compound 48/80 mediated histamine release. B) Histamine release triggered by Substance P. C) Histamine release evoked by FcεRI-aggregation. ** $p < 0.01$; **** $p < 0.0001$ significantly higher or lower from control (paired t-test).

Author contribution Design: MB; experiments: SG, MA; data analysis/writing: MB. Manuscript revision: TZ. All authors approved this final version.

Funding

Supported by ECARF and GA²LEN, aided by the Sixth EU Framework programme (FOOD-CT-2004-50637).

Disclosure of potential conflict of interest

The authors declare they have no relevant conflicts of interest.

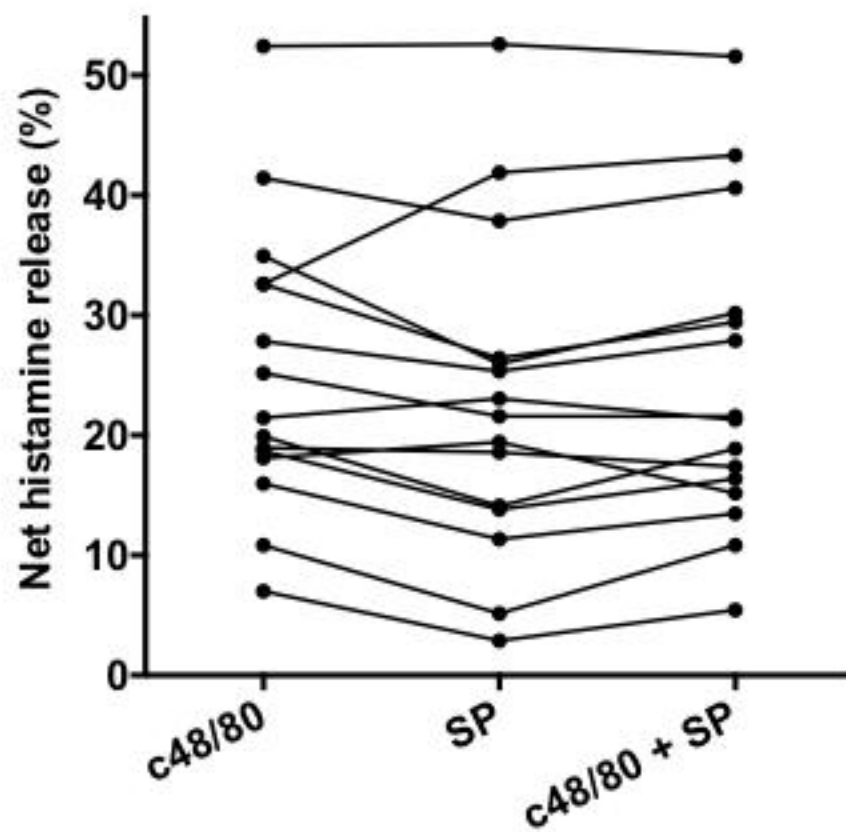
Acknowledgement

We thank Ms. von Grüner and Ms. Heßler for excellent technical assistance.

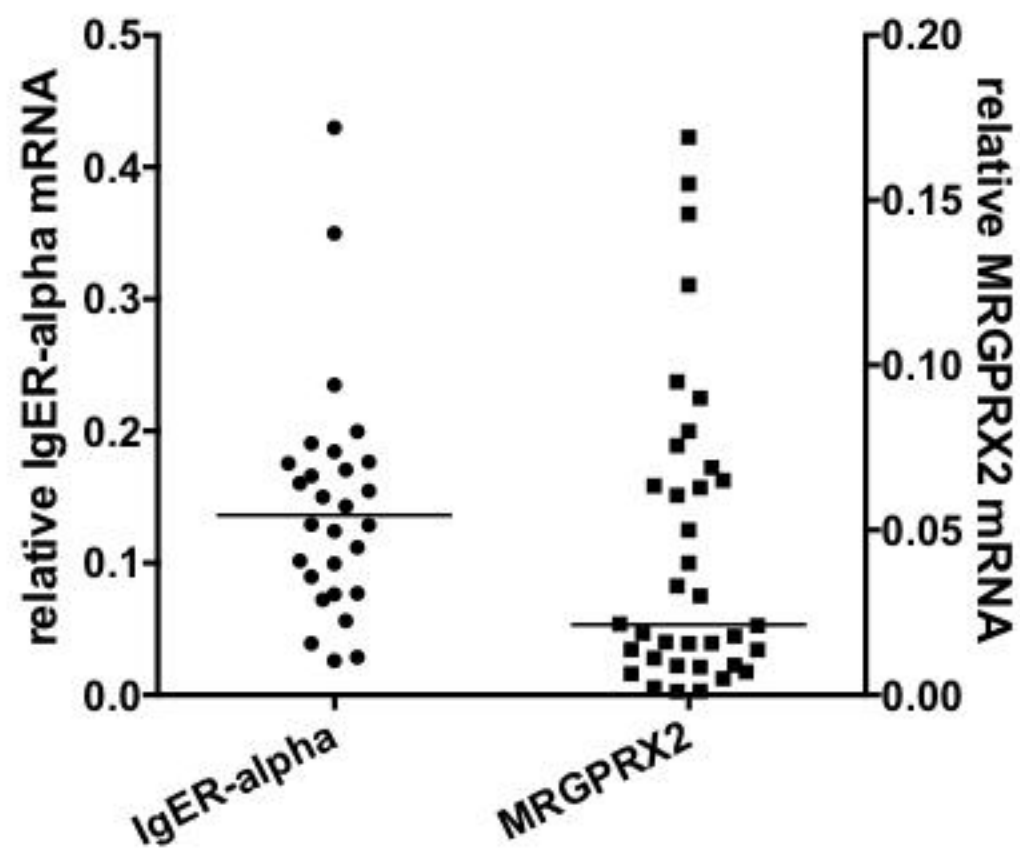
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Suppl. Figure 1



Suppl. Figure 2

Supplementary

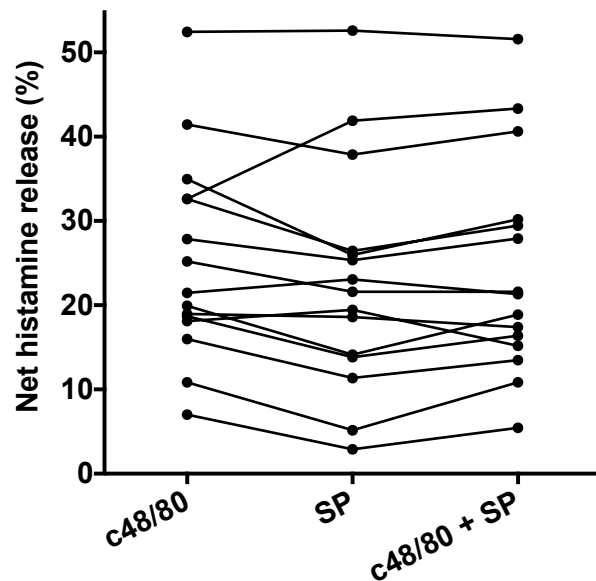


Figure E1

Histamine responses are not increased when c48/80 and SP are provided simultaneously versus individually. Activation by compound 48/80 (10 μ g/ml) and Substance P (30 μ M) and the combination of the two in n=15 MC preparations (donors).

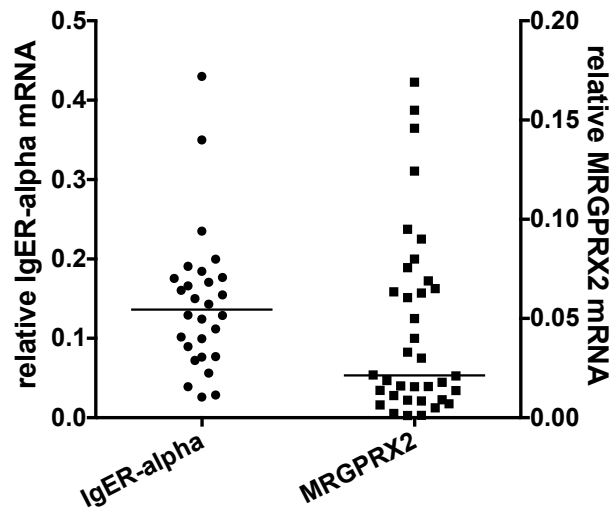


Figure E2

Substantial variation in MRGPRX2 mRNA expression levels across individuals

IgER-alpha ($\text{Fc}\epsilon\text{RI}\alpha$) is given for comparison; relative mRNA normalized to β -actin is given and each dot depicts one individual. $n = 28$ for $\text{Fc}\epsilon\text{RI}\alpha$, plotted on left y-axis, $n = 36$ for MRGPRX2, plotted on right y-axis. The bar shows the median. The coefficients of variation are as follows: 61.3% for $\text{Fc}\epsilon\text{RI}\alpha$, 102.9% for MRGPRX2.