

RESEARCH LETTER

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Tryptase release does not discriminate between IgE- and MRGPRX2-mediated activation in human mast cells

To the Editor,

Since the seminal description of the Mas-related G protein-coupled receptor X2 (MRGPRX2) potentially being involved in immediate drug hypersensitivity reactions (IDHRs), this hypothesis has gained significant momentum.¹ However, important knowledge gaps and uncertainties remain and diagnosis of MRGPRX2-dependent IDHRs continues to be challenging, mainly because of the absence of a reference test to unambiguously document such reactions. In an attempt to advance insights in MRGPRX2-dependent IDHRs, we retrospectively compared clinical and biological data from patients who experienced anaphylaxis to rocuronium and were *post hoc* stratified as most likely sIgE- and possible MRGPRX2-dependent. It appeared that both endotypes display similar clinical details, acute tryptase and delta tryptase levels (acute tryptase level minus baseline tryptase level).² Nevertheless, based upon the mechanistic differences between IgE- and MRGPRX2-dependent mast cell (MC) degranulation as described by Gaudenzio et al,³ we thought it was meaningful to continue exploration of tryptase as a potential biological discriminator. Actually, our method to classify patients as possibly MRGPRX2-reactors might not find universal acceptance and *in vitro* findings about the MRGPRX2 agonistic activity of rocuronium are not unanimous. For example, we demonstrated that rocuronium, despite an increase in intracellular calcium, does not trigger degranulation *in vitro* as reflected by up-regulation of the lysosomal degranulation marker CD63.⁴ Therefore, as a first set of experiments, we compared tryptase release from human peripheral blood cultured mast cells (PBCMCs) of healthy controls in response to stimulation with known potent secretagogues, that is, recombinant major birch pollen allergen (rBet v 1) and substance P, respectively, representative for sIgE- and MRGPRX2-dependent MC activation.

Peripheral blood cultured mast cells, defined as CD117⁺CD203c⁺, were cultured from peripheral blood progenitor cells, as previously described.^{5,6} Briefly, CD34⁺ progenitor cells were isolated using the EasySep Human CD34 Selection Kit out of peripheral blood mononuclear cells according to the manufacturer's instructions. Isolated CD34⁺ progenitor cells were cultured in a serum-free methylcellulose-based medium supplemented with low-density lipoprotein (LDL, 10 µg/mL), 2-mercaptoethanol (55 µmol/L), stem cell factor (SCF, 100 ng/mL), interleukin-3 (IL-3, 100 ng/mL) and interleukin-6 (IL-6, 50 ng/mL) for 4–5 weeks. Participants gave written informed consent, and the study was approved by the Ethical Committee of the University Hospital of Antwerp (B300201837509).

Mast cell activation and degranulation were measured by the quantification of the up-regulation of the density of the ectoenzyme CD203c and percentage up-regulation of CD63, respectively. As shown earlier, interleukin-33 (IL-33) potentiates MC activation/degranulation via cross-linking of the membrane-bound IgE/FcεRI complexes.^{7,8} Therefore, for further functional evaluation of sIgE/FcεRI pathway, cells were systematically primed with IL-33. In contrast, IL-33 was confirmed not to affect MRGPRX2-mediated activation.⁸ For the IgE-dependent degranulation, PBCMCs (concentration of 5×10^5 cell/mL) were passively sensitized overnight with serum from birch pollen-allergic patients sensitized to Bet v 1, the major allergen from birch pollen (*Betula verrucosae*), in a 1:1 ratio, at 37°C in a humidified CO₂-incubator. Titers of sIgE rBet v 1 varied between 2.06 and 8.67 kUA/L (normal <0.10 kUA/L, FEIA ImmunoCAP, Phadia Thermo Fisher Scientific, Uppsala, Sweden). All patients presented with rhinoconjunctivitis and/or asthma related to birch pollen exposure. Next, sensitized PBCMCs were centrifuged (500 g, 5 min, 20°C) and the cell pellet was resolved in pre-warmed Tyrode's buffer at a concentration of 5×10^5 cells/mL. Thereafter, 100 µL of the sensitized PBCMCs were pre-incubated with IL-33 (100 ng/mL) for 20 min at 37°C. Subsequently, the pre-incubated PBCMCs were stimulated with 100 µL of Tyrode's buffer as a negative control or recombinant Bet v 1 (rBet v 1—final concentration of 0.05 µg/mL) as allergen for 20 min at 37°C. For IgE-independent stimulation, PBCMCs were suspended in pre-warmed (37°C) Tyrode's buffer at a concentration of 5×10^5 cells/mL. Next, 100 µL of the cells were stimulated with 100 µL Tyrode's buffer as a negative control or 100 µL substance P (final concentration of 74 µmol/L) during 3 min at 37°C. Reactions were stopped by placing the cells on ice. Subsequently, supernatants were collected after centrifugation (500 g, 5 min, 4°C) and stored at –20°C. Cells were resuspended in PBS supplemented with 0.1% BSA and stained with monoclonal anti-human CD117-APC, anti-human CD203c-PECy7, anti-MRGPRX2-PE and anti-human CD63-FITC for 20 min at 4°C in the dark. Finally, cells were washed and resuspended in PBS_NaN3 and analysed using a FACSCanto II™ flow cytometer and Kaluza Analysis 2.1 software. Tryptase levels in the collected supernatants were determined using ImmunoCAP™ according to the manufacturer's instructions.

As shown in Figure 1, for sIgE-mediated activation, pre-incubation with IL-33 resulted in an up-regulation of CD203c without up-regulation of CD63 nor significant release of tryptase. In contrast, activation with rBet v 1 induced an up-regulation of the activation marker CD203c and degranulation, as reflected by the up-regulation

of the degranulation marker CD63 together with a quantifiable release of tryptase. Activation with substance P, the natural ligand of MRGPRX2, also resulted in a significant up-regulation of CD203c and CD63, accompanied by a release of tryptase. Importantly, as shown in Figure 1, no difference in the delta tryptase (tryptase level after activation minus tryptase level before activation) between sIgE- and MRGPRX2-mediated degranulation was demonstrable.

In a second part of the study, focussing on PBCMC responsiveness to MRGPRX2 agonists, we quantified tryptase in the supernatants of earlier studies with morphine and rocuronium.⁴ Activation was performed as described above. PBCMCs were stimulated with morphine (final concentration of 500 $\mu\text{mol/L}$) or rocuronium (final concentration of 1680 $\mu\text{mol/L}$). Final concentrations were determined upon previous dose-finding experiments.⁴

Like substance P, morphine induced an up-regulation of CD203c, CD63 and a significant release of tryptase. Of note, substance P and morphine caused comparable tryptase release. In contrast,

Key messages

- Tryptase release is not discriminative between IgE- and MRGPRX2-mediated human mast cell activation.
- Tryptase release mirrors degranulation, as reflected by CD63 up-regulation.
- Further investigation of potential discriminative biomarkers will necessitate human mast cells.

rocuronium induced a small MRGPRX2-dependent increase in intracellular calcium⁴ but this was not accompanied with quantifiable release of tryptase (Figure 1). Representative flow cytometric plots of CD203c and CD63 are shown in Figure 2.

Taken together, we demonstrate that sIgE- and MRGPRX2-mediated degranulation of human PBCMCs, as reflected by an

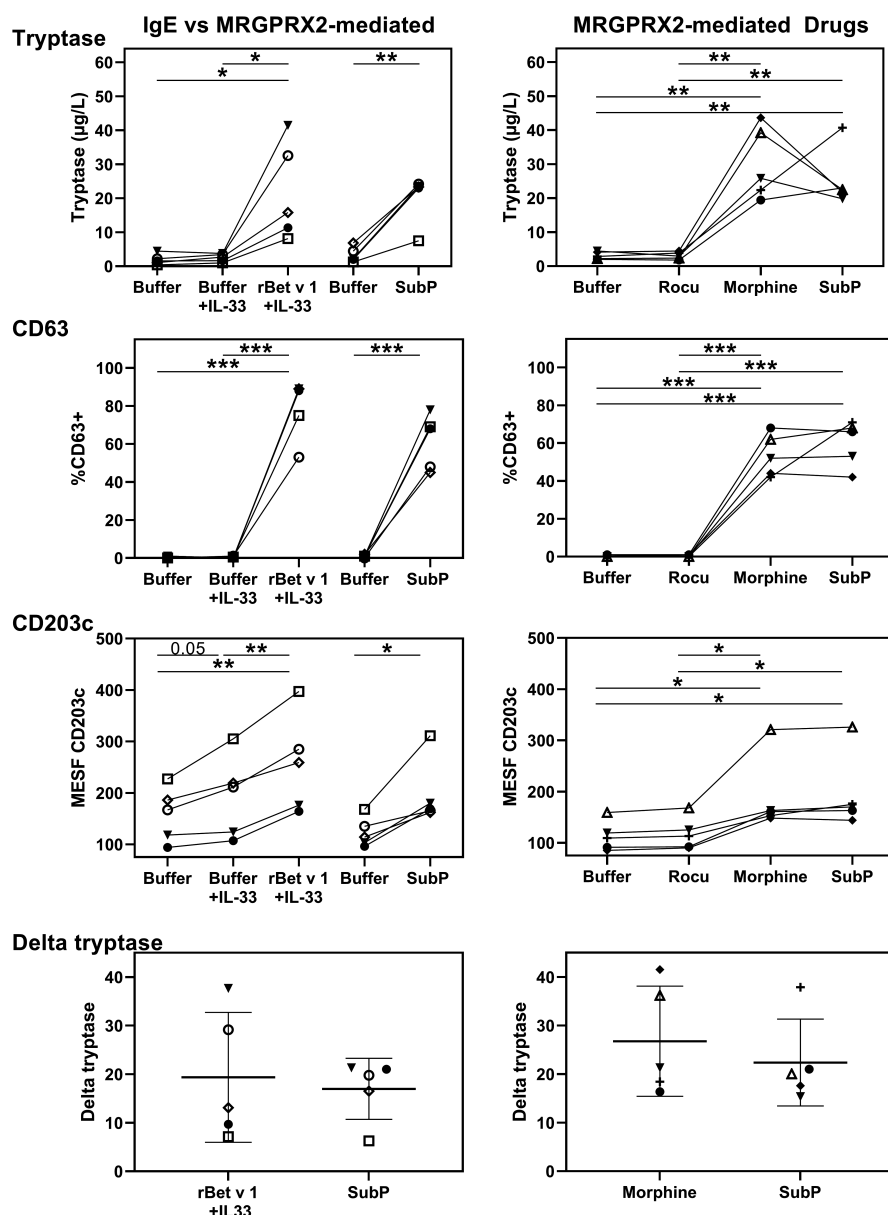
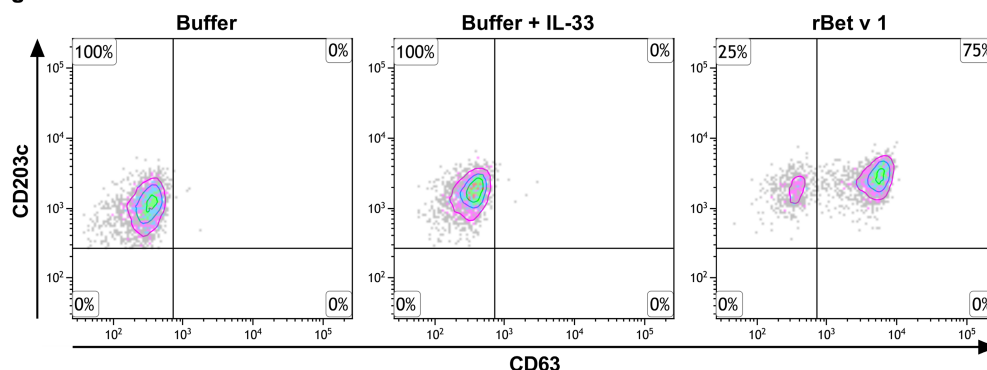


FIGURE 1 Tryptase levels, delta tryptase, up-regulation of percentage CD63 and density of CD203c after sIgE- or MRGPRX2-mediated activation. Peripheral blood cultured mast cells (PBCMCs) were activated via a sIgE-mediated mechanism with recombinant major birch pollen allergen rBet v 1 (0.05 $\mu\text{g/mL}$) or via a MRGPRX2-mediated pathway with substance P (74 $\mu\text{mol/L}$). PBCMCs were also incubated with the neuromuscular blocking agent rocuronium (1680 $\mu\text{mol/L}$) and the opiate morphine (500 $\mu\text{mol/L}$). Tryptase release, CD63 up-regulation, density of CD203c and delta tryptase. Delta tryptase is calculated as the tryptase level after activation minus the tryptase level before activation. Figure displays individual values with mean $\pm$ SD. Paired Student's *t*-tests: $p < 0.05^*$, $p < 0.01^{**}$, $p < 0.001^{***}$. In all experiments, $n = 5$. Each symbol in the figures denotes a PBCMC culture. SubP, substance P; rocu, rocuronium

slgE-mediated



MRGPRX2-mediated

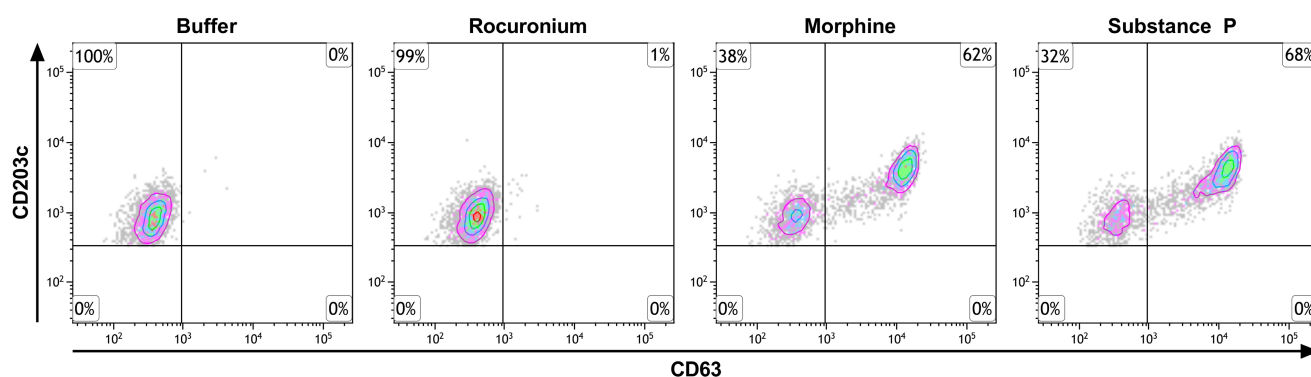


FIGURE 2 Representative plots of CD203c and CD63 up-regulation after a slgE- or MRGPRX2-mediated activation of the MCs. Peripheral blood cultured mast cells (PBCMCs) were activated via a slgE-mediated mechanism with recombinant major birch pollen allergen rBet v 1 (0.05 $\mu\text{g/mL}$) or via a MRGPRX2-mediated pathway with substance P (74 $\mu\text{mol/L}$), the neuromuscular blocking agent rocuronium (1680 $\mu\text{mol/L}$) and the opiate morphine (500 $\mu\text{mol/L}$)

up-regulation of CD63, results in a release of tryptase with similar magnitude. Admittedly, our study has some limitations. Quantitative comparison with total tryptase content of cells and larger series would have added more rigour to our conclusions. However, these in vitro findings align with earlier in vivo findings, viz, baseline, acute and delta tryptase being non-discriminative between both pathways of MC degranulation. The difference with the findings of Meixiong et al.⁹ is likely related to the adaptive evolution of MRGPRX2, which makes once again the extrapolation of murine data into the human situation difficult.¹⁰ Consequently, further searches of potential discriminative biomarkers will necessitate human mast cells.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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