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ABSTRACTS

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Role of Tyrosine residues in MRGPRX2 on G protein and β-arrestin-mediated signaling in response to substance P

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Recent evidence suggests that substance P (SP) contributes to neurogenic inflammation, chronic idiopathic urticaria and atopic dermatitis through mast cell activation via Mas-related G protein-coupled receptor (GPCR)-X2 (MRGPRX2). We found that SP serves as a balanced agonist for MRGPRX2 as it activated both G protein and β-arrestin-mediated signaling pathways. MRGPRX2 contains six tyrosine residues, five located in transmembrane domains and one in the intracellular loop 2. However, the role of MRGPRX2's tyrosine residues on G protein and β-arrestin-mediated signaling is unknown. To address this question, we prepared cDNA encoding MRGPRX2 mutants; Y67A, Y89A, Y113A, Y137A, Y222A and Y279A, and generated transient transfectants in RBL-2H3 cells. We found that cells expressing Y67A, Y113A, Y137A or Y279A mutant were unresponsive to SP-induced Ca^{2+} mobilization and degranulation. Tyrosine Y279 in MRGPRX2's seventh transmembrane domain within the NPXXY motif is important for G protein coupling. To determine if Y279 also contributes to β-arrestin-mediated signaling, we generated Y279A mutant in a plasmid used for transcriptional activation following arrestin translocation (TANGO) assay, and transiently transfected in engineered HEK293 cells (HTLA). We found that Y279A mutation resulted in significant loss of β-arrestin-mediated gene expression and receptor internalization in response to SP when compared to the wild-type receptor. These findings provide the first demonstration that a single tyrosine residue of MRGPRX2 may be responsible for both G protein and β-arrestin-mediated signaling.

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MRGPRX2 and immediate drug hypersensitivity: insights from cultured human mast cells

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Background: Mast cell (MC) degranulation via off-target occupation of Mas-related G protein coupled receptor X2 (MRGPRX2) heralds a new domain in our knowledge of immediate drug hypersensitivity (IDH). However, findings and conclusions are not unanimous and today data in humans are limited to observations in specific cell lines.
Aim: To study silencing MRGPRX2 of cultured human MC as a tool to further unveil the MRGPRX2 pathway in IDH.
Methods: MCs are cultured out of CD34+ progenitor cells obtained from peripheral blood (PBCMCs) and incubated with substance P, succinylcholine, atracurium, ciprofloxacin or levofloxacin. Intracellular calcium is measured using Fluo-4. Degranulation is analysed by quantification of CD63 expression. For MRGPRX2 silencing, PBCMCs are electroporated with Dicer-substrate silencing RNAs.
Results: Incubation of PBCMCs with substance P, atracurium, ciprofloxacin and levofloxacin results in an increase in intracellular calcium and degranulation that is restricted to the MRGPRX2+ cells. Both phenomena are strongly mitigated via selective MRGPRX2 silencing. Succinylcholine has no effect on PBCMCs. Silencing of MRGPRX2 did not affect the IgE-dependent activation.
Conclusion: MRGPRX2 silencing in PBCMC can deepen our insights and shift paradigms about the mechanisms that govern MC degranulation via off-target occupancy of MRGPRX2 by drugs.

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Validation of a novel immunomodulatory compound for restoring tolerance

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Present treatments of allergies are mainly aimed at symptomatic treatment rather than curing patients. Specific-immunotherapy can achieve this, but comes with limitations including low compliance and local or systemic side effects such as asthma attacks and life-threatening anaphylactic shock. Bioactive compounds that restore tolerance by inducing functional regulatory T cells (Tregs) may overcome these limitations, but are not available yet.
By high-throughput screening, we identified one compound (C5) significantly upregulating the expression of Foxp3 (a master transcription factor of Tregs). We validated C5 in dose response studies with primary human $CD4^+$T cells, analyzed toxicity, proliferation, and the production of IL-4 and IFN-y by C5–treated $CD4^+$ T cells. Furthermore, we performed suppression assays using C5-treated $CD4^+$ memory T cells.
C5 significantly increased Foxp3 expression in primary human naïve and memory $CD4^+$T cells (50% and 69% respectively), without impairing cell proliferation and viability. In addition, C5 treatment decreased the frequencies of IL-4-producing total memory and $Foxp3^+CD4^+$T cells while frequencies of IFN-y producing cells increased. Importantly, C5-treated memory T cells reduced the proliferation of conventional $CD4^+$T cells.
C5 might be a potential therapeutic candidate to restore immune tolerance by converting conventional $CD4^+$ T cells into suppressive Foxp3-expressing T cells in allergies.

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Revisiting the Effect of Cromolyn Sodium on Human and Murine Mast Cells

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Background: Cromolyn sodium (CS) has been extensively described as a mast cell (MC) stabilizer for its ability to impair histamine release in human, murine and rat MCs. Because of this effect, it was employed as an anti-allergy drug. However, CS' mechanism of action is still poorly explained.
Aims: We aimed to further characterized CS effect *in vitro* on human cord blood-derived MCs (CBMCs) and *in vivo* in murine models of allergic inflammation.
Methods: CBMCs were incubated with CS and activated via IgE-mediated activation. Pro-inflammatory and pro-resolution effectors were investigated. Allergic peritonitis (AP) was induced in Wild Type (WT) mice by OVA/SEB subcutaneous sensitization and OVA/SEB intraperitoneal challenge. Asthma was induced in WT mice by intranasal house dust mite (HDM) sensitization and challenge. CS was administered daily after challenge. Mice were euthanized at select time points and inflammation parameters were analysed.
Results: CS did not control tryptase or IL-8 release from human CBMCs, but expression of the anti-inflammatory receptor CD300a and IL-10 were increased. In the AP model, CS treatment significantly reduced total cells and eosinophil (Eos) numbers and increased IL-10 peritoneal levels. Similar to AP, HDM-induced allergic lung inflammation was decreased by CS with fewer total cells and Eos.
Conclusions: These results point to an anti-inflammatory, rather than stabilizing, action for CS via modulation of MC pro-resolutory effectors.

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Prostaglandin D_2 inhibits mediator release and antigen induced bronchoconstriction in the guinea pig trachea by activation of DP_1 receptors

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Background:
Unselective cyclooxygenase (COX) enzyme inhibition causes an amplification of antigen-induced contractions in guinea pig and human airways. However, the mechanism behind this is still unknown.
Methods:
Male albino guinea pigs were sensitised to ovalbumin (OVA). After 14 days, tracheal segments were dissected out and mounted in organ baths to asses smooth muscle contractility and analyse lipid mediator release with UPLC-MS/MS or EIA following certain pharmacological interventions.
Results:
OVA challenge increased the release of prostanoids (prostaglandin (PG) D_2/E_2/$F_{2\alpha}$/I_2 and thromboxane (TX) A_2). Unselective COX-inhibition abolished release of all prostanoids, whereas COX-2 inhibition blocked release of all prostanoids except PGD_2 and TXA_2. Leukotriene B_4 and E_4 levels were increased by OVA and further increased after unselective COX-inhibition. Furthermore, when selectively inhibiting prostaglandin D synthase (PPCA), antigen-induced bronchoconstriction was amplified, an action that was reversed by exogenous PGD_2. Moreover, a DP_1 receptor agonist (BW 245c) concentration-dependently reduced the antigen-induced constriction, as well as reducing released histamine and CysLTs. In contrast, a DP_2 receptor agonist (15(R)-15-methyl PGD_2) had no effect on OVA-induced constrictions.
Conclusion:
PGD_2 is formed by COX-1 and via the DP_1 receptor exerts an inhibitory effect on antigen-induced bronchoconstriction, most likely by inhibition of mast cell activity.

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Cyclic hypoxia promotes a hyperresponsive phenotype to FcεRI crosslinking in MCs

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The role of Mast Cells (MCs) on tumor growth is far from being understood. Evidence indicates that MCs infiltrate solid tumors and execute pro- or anti-tumorigenic functions depending on tumor type, MCs localization, and conditions present in the tumor microenvironment (TME). Since cyclic hypoxia (CyH), a distinctive feature of almost all solid tumors, could play a role in regulating the phenotype of intratumoral MCs, we analyzed changes in the transcriptome of MCs exposed to CyH by microarrays assay. Bone marrow-derived mast cells (BMMCs) were subjected to CyH in a protocol composed by several cycles of hypoxia and re-oxygenation. The increase on HIF-1α and VEGF-A mRNAs, together with the production of reactive oxygen species (ROS) were determined as a control of CyH. Importantly, we observed the upregulation of genes related to the high-affinity IgE receptor (FcεRI) signaling pathway. Accordingly, FcεRI-dependent degranulation was exacerbated in BMMCs under CyH compared to those maintained in normoxia. For the first time, these findings link CyH to MCs phenotype plasticity and suggest that CyH could act as a positive modulator of MCs-activation in the TME, which should be considered in the design of therapies targeting inflammation to control tumor growth.