

LETTER

Mast cells derived from systemic mastocytosis exhibit an increased responsiveness to hyperosmolarity

To the Editor,

Systemic mastocytosis (SM) is a disease characterized by increased number of aberrant mast cells in one or several organs and increased systemic levels of mast cell (MC) mediators.¹ Indolent SM (ISM) is the most common form of SM, constituting approximately 80% of the patients diagnosed with SM. Individuals with ISM often have mediator mediated symptoms, most commonly from the skin, the gastrointestinal tract, cardiovascular, and respiratory system, but also in the form of anaphylaxis. Although basal mediator levels, including serum tryptase and metabolites of histamine and prostaglandin D₂ in the urine, are increased at steady state,¹⁻³ the symptoms often come as spells without any obvious trigger suggesting an intrinsic defect causing a hyper-reactive state of the mast cells, or an endogenous trigger.

We have previously addressed the hypothesis of a hyper-reactive MC phenotype in ISM by *in vivo* provocation.² None of the used triggers mounted a response that was different between ISM patients and healthy volunteers (HV). To further investigate the hypothesis of a hyper-reactive MC phenotype, we also developed MCs *in vitro* from 14 ISM patients and 13 HV (same subjects as included in²) (Tables S1 and S2). Peripheral blood was obtained, and CD34-selected progenitor cells were cultured under MC promoting conditions⁴ (see supplement). When the cells were mature, they were plated and exposed to IgE-receptor activation, morphine, or mannitol-induced hyperosmolarity, representing three distinct activation pathways (see supplement). Histamine (as a measurement of degranulation) and PGD₂ (newly synthesized lipid mediator); that is, two prominent MC mediators, released through two different routes that are increased in ISM, were measured.

We did not observe any difference in *in vitro* growth and development of MCs over a 6-week period between cells from ISM patients and HV (Figure 1). This result stands in contrast to a study where a significant increase in MC growth from CD34-selected progenitor cells from ISM patients was described.⁵ An explanation could be the different culture protocols used in the two studies.⁵ The *in vitro* developed MCs (>90% tryptase positive) were plated and exposed to different MC secretagogues: the calcium ionophore A23187, morphine, anti-IgE, and mannitol. The release of histamine

was comparable between MCs derived from ISM and HV in response to all tested secretagogues (Figure 2A). In contrast, MCs derived from ISM showed a significantly increased release of PGD₂ in response to mannitol, but not to the other tested triggers (Figure 2B). It has been reported previously that the release of β -hexosaminidase (released through degranulation) after IgE-receptor activation is the same from MCs derived from ISM as from HV.⁵ However, in that study, they neither investigated the secretion of PGD₂, nor other type of secretagogues.

Our study provides the first evidence that MCs derived from ISM exhibit an aberrant response profile to mannitol-induced hyperosmolarity, with no change in degranulation but an increased synthesis and secretion of PGD₂, the main eicosanoid produced by MCs. Mannitol is clinically used to measure bronchoconstriction in individuals with asthma. Individuals with mastocytosis have not been reported to have increased risk for asthma and airway hyperresponsiveness, and in our previously published study, we did not observe any increased bronchoconstriction after mannitol challenge in those with mastocytosis.² Here, we used mannitol as a stimulus to mimic

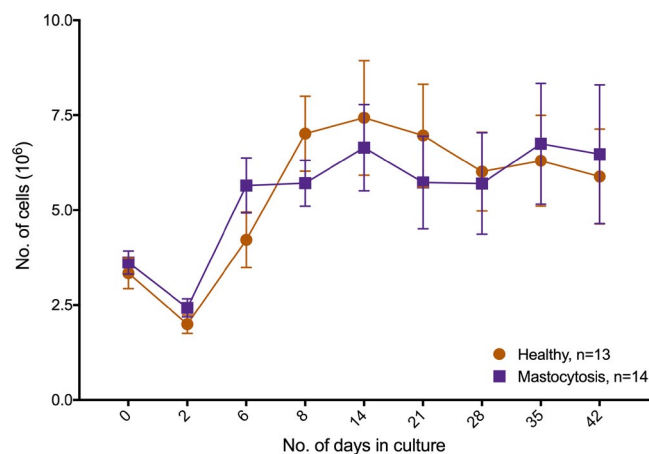


FIGURE 1 *In vitro* growth and maturation of cells over a 42-day period. CD34-selected peripheral blood cells from healthy volunteers ($n = 13$) or individuals with indolent systemic mastocytosis ($n = 14$) were cultured under conditions that promote mast cell development. Mean $\pm$ SEM

Theo Gülen Shared last authorship.

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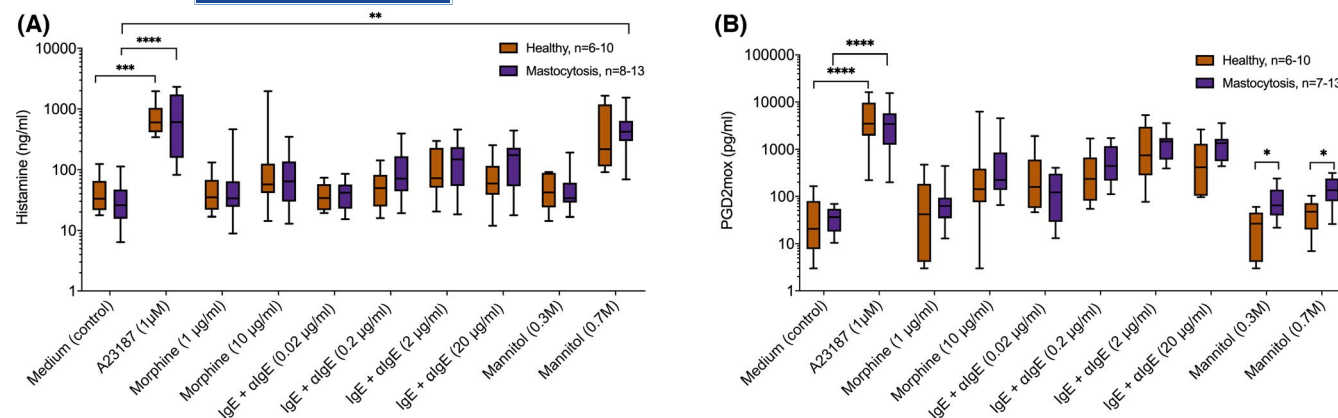


FIGURE 2 Release of histamine and PGD₂ from activated *in vitro* developed mast cells. Mast cells were treated for 30 minutes with calcium ionophore A23187, morphine, anti-IgE, or mannitol, and the release of histamine (A) and PGD₂ (B) was measured in the cell free supernatant. Healthy volunteers (red boxes) ($n = 6-10$) and systemic mastocytosis (purple boxes) ($n = 7-13$). Results are shown as box and whiskers; the box extends from the 25th to 75th percentiles and the whiskers min to max. * $p < 0.05$

hyperosmolarity. Cells sense physical changes through the receptor family transient receptor potential vanilloid type 1-4 (TRPV).⁶ Thus, one could speculate that MCs sense osmolarity changes through TRPV and that this pathway is altered in mastocytosis patients, resulting in an increased synthesis and release of PGD₂.

A hyper-reactive MC phenotype in ISM is still elusive, but our data indicate that an intrinsic defect in these cells could affect other signaling pathways than the commonly studied downstream of the IgE-receptor and that other mediator releasing systems than degranulation, that is, newly synthesized mediators, should be studied.

KEYWORDS

histamine, IgE, mast cells

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CONFLICT OF INTEREST

The authors report no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

Supplement

Materials and methods

Study subjects

Individuals with indolent systemic mastocytosis (n=14) and healthy volunteers (n=13) were recruited to the Mastocytosis center Karolinska. The patients with systemic mastocytosis fulfilled the WHO criteria for indolent systemic mastocytosis. Demographic data and characteristics of the study subjects are shown in table S1 and table S2. The Ethical Review Board in Stockholm approved the study (Dnr 2009/959-31-4), and subjects provided written consent.

In vitro development of mast cell

Mononuclear cells were extracted from a 450 ml blood donation by centrifugation on Ficoll-Paque and the CD34 positive cell population was enriched using magnetic bead separation according to the manufacturers protocol (Miltenyi). The CD34-enriched cells were cultured using a protocol described by Lappalainen et al.⁵. 0.5×10^6 /ml cells were plated in serumfree media, StemSpan (R&D systems), supplemented with Penicillin and Streptomycin 100U/ml (Sigma-Aldrich) and SCF 100 ng/ml (kindly provided by SOBI, Stockholm, Sweden). Low density lipoprotein LDL 10 μ g/ml was added to the culture from day 0 till day 35. Cytokines (Peprotech) were supplemented at different timepoints; IL-3 was added to the culture from day 0 until day 8, IL-6 was in the culture from day 35 and onward, IL-9 only at day 8 -14 and IL-4 was added to the culture 3 days prior to cell activation. The first four weeks the cells were cultured under hypoxic conditions with 5% oxygen and then moved to normoxia with 21% oxygen at 37°. The cell number and viability were counted using trypan blue exclusion and level of tryptase-positive cells were investigated using enzymatic staining.

Mast cell activation

For IgE-receptor activation the cells were preincubated with IL-4 (10 ng/ml) for 72 h and then washed and incubated with human IgE (1 μ g/ml) for 24h (Calbiochem). After washing the cells were suspended in PIPES buffer and activated at 37°C for 30 min using Calcium ionophore A23187 (1 μ M), morphine (1 μ g/ml), anti-IgE (0.02-20 ng/ml) and mannitol (0.3 and 0.7 M) (Sigma-Aldrich). The activation was stopped by putting the tubes on ice before the cells were spun down and supernatants stored at -70°C.

Analysis of Histamine and Prostaglandin D₂

The collected supernatants were analyzed for histamine using microtiter plates coated with glass microfibers where the adsorbed histamine was fluorometrically detected using the Histareader (RefLab Aps, Copenhagen). PGD₂ was measured by a competitive enzyme immunoassay according to the manufacturer's instructions (Cayman Chemical, Ann Arbor, USA), PGD₂ is converted to PGD₂-MOX by treatment with methoxylamine hydrochloride and PGD₂-MOX is recognized by a rabbit antisera in competitive ELISA.

Statistical analysis

The cell proliferation was analyzed using 2-way ANOVA with Bonferroni's posttest while mediator release was compared using student T-test and one-way ANOVA with Dunnett's posttest.

Table S1. Demographic data and characteristics of the study subjects

	<i>Indolent Systemic Mastocytosis (n=14)</i>	Healthy volunteers (n=13)
Age, years <i>median (range)</i>	51 (32-69)	36 (22-55)
Sex, M/F	5/9	5/8
D816V mutation in bone marrow	13/14	0
Total IgE kE/l, <i>median (range)</i>	15 (2-140)	17 (6.9-36)
Serum tryptase µg/L, <i>median (range)</i>	27.5 (13-320)	5.3 (1-15)

Table S2. Description of patients with systemic mastocytosis

Pat. Nr, Sex	Age at Dx	Skin lesion	History of Ax	Elicitor of Ax	Clinical pattern of Ax	Presence of AD	MC clusters	D816V mutation	sBT (μ g/L)
1, F	46	ISMs ⁺	No	n/a	n/a	No	No	Positive	26
2, F	43	ISMs ⁺	Yes	Unknown	Flush, syncope, dyspnea,	Asthma	Yes	Positive	15
3, M	53	ISMs ⁺	Yes	Unknown	Flush, syncope, dyspnea,	No	No	Positive	25
4, M	64	ISMs ⁺	Yes	Wasp, SPT ⁺ /CAP ⁺	Flush, syncope, dyspnea,	No	Yes	Negative	29
5, F	68	ISMs ⁺	Yes	Wasp, SPT ⁺ /CAP ⁺	Sync	No	No	Positive	15
6, F	63	ISMs ⁺	Yes	Shrimp, SPT ⁺ /CAP ⁺	Flush, dyspnea, syncope, vomit	No	Yes	Positive	36
7, F	68	ISMs ⁺	No	n/a	n/a	No	Yes	Positive	81
8, F	69	ISMs ⁺	No	n/a	n/a	No	Yes	Positive	320
9, M	43	ISMs ⁻	Yes	Wasp, SPT ⁺ /CAP ⁺	BP-fall, dyspnea	Asthma	No	Positive	13
10, F	42	ISMs ⁺	No	n/a	n/a	No	Yes	Positive	217
11, F	49	ISMs ⁺	No	n/a	n/a	Asthma	Yes	Positive	120
12, F	32	ISMs ⁺	No	n/a	n/a	No	No	Positive	18
13, M	45	ISMs ⁻	Yes	Unknown	Flush, syncope	No	Yes	Positive	160
14, M	65	ISMs ⁺	Yes	Wasp, SPT ⁺ /CAP ⁺	BP-fall, syncope	Asthma	No	Positive	21

ABBREVIATIONS: Dx, diagnosis; Ax, anaphylaxis; sBT, serum baseline tryptase; MC, mast cell; AD, atopic disease; F, female; M, male; ISMs⁻, indolent systemic mastocytosis without skin engagement; ISMs⁺, indolent systemic mastocytosis with skin engagement; SPT, skin prick test; CAP, ImmunoCAP specific IgE; n/a, non-applicable

