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SUPPORTING INFORMATION

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

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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 D_2 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 in[2]) (Tables S1 and S2). Peripheral blood was obtained, and CD34-selected progenitor cells were cultured under MC promoting conditions[4] (see supplement). When the cells were mature, they were plated and exposed to IgE-receptor activation, morphine, or

Theo Gülen Shared last authorship.

mannitol-induced hyperosmolarity, representing three distinct activation pathways (see supplement). Histamine (as a measurement of degranulation) and PGD_2 (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 PGD_2 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 PGD_2, 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 PGD_2, 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 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 PGD_2.

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.

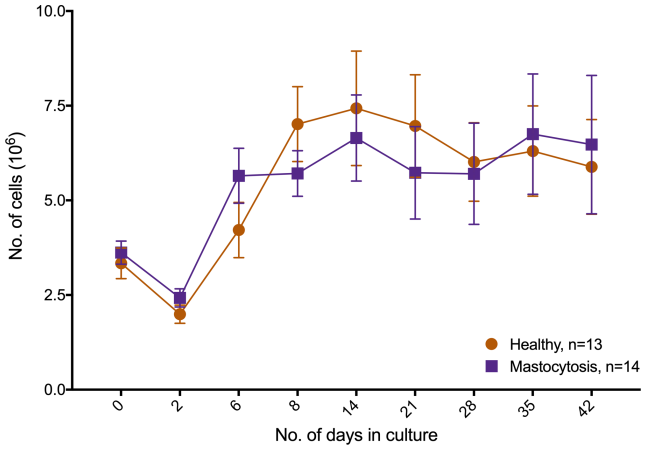

FIGURE 1 *In vitro* growth and maturation of cells over a 42-day period. CD34-selected peripheral blood cells from healthy volunteers ($n = 13$) or individuals with indolent systemic mastocytosis ($n = 14$) were cultured under conditions that promote mast cell development. Mean ± SEM

KEYWORDS

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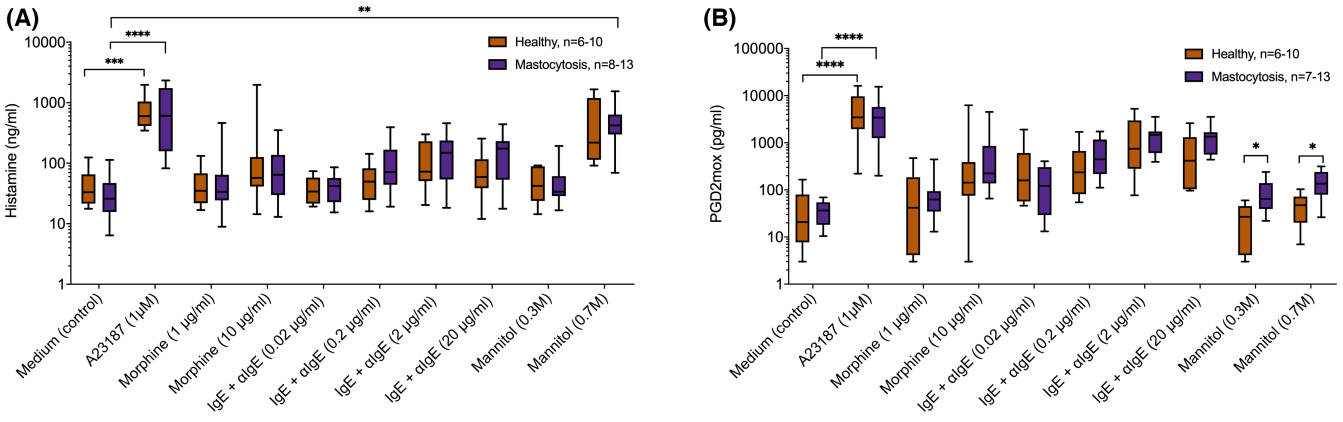

FIGURE 2 Release of histamine and PGD_2 from activated *in vitro* developed mast cells. Mast cells were treated for 30 minutes with calcium ionophore A23187, morphine, anti-IgE, or mannitol, and the release of histamine (A) and PGD_2 (B) was measured in the cell free supernatant. Healthy volunteers (red 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. * $p < 0.05$

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CONFLICT OF INTEREST

The authors report no conflict of interest.

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SUPPORTING INFORMATION

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Single-cell transcriptomics of mouse lung reveal inflammatory memory neutrophils in allergic asthma

To the Editor,

Neutrophilic asthma is associated with increased disease severity and poor response to glucocorticosteroids, but the role of neutrophils in asthma remains controversial.[1] Innate immune memory, also known as trained immunity, refers to the enhanced immune responsiveness of primed innate immune cells under secondary stimulation, and is characterized by epigenetic reprogramming of cells mediated by transcription factors (TFs). Innate immune memory regulates both inflammation and immunological tolerance,[2] which may play an important role in airway inflammation and allergic asthma. Neutrophils are associated with innate immune memory, but the subpopulation of memory neutrophils and their markers have not been defined yet.

In this study, we performed single-cell RNA sequencing (scRNA-seq) on lung tissues obtained from mice with ovalbumin (OVA)-induced chronic allergic asthma (See Appendix S1 for detailed